10-21-82

UNITED ST. .NMENTAL PROTECTION AGENCY
WASHI..GTON, D.C. 20460

MEMORANDUM

002256

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Franklin D. Gee, Product Manager #17
Registration Division (TS-767)

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: Mavrik® (fluvalinate). (1) Review of Animal Studies Conducted with Racemic or Half-Resolved Fluvalinate as Formulated or as Technical Material. (2) Review of Human Exposure Reports.

TOX Chem. No. 934

The following questions have been raised or additional information requested or points of clarification needed with respect to the following studies:

Acute Oral LD50 - Rat. Accession No. 070664. Study No. WIL-80203.

1. What is the white precipitate a sign of in the mouth of the rats?

2. Would the cause of this sign affect the results of the study?

3. We ask the petitioner to address the signs "scratching and digging" in this study in light of the scratching phenomenon observed in other studies, as well as the appearance of skin lesions in other studies (i.e., rats and dogs).

Acute Oral LD50 - Rat. Acute Oral LD50 - Mouse. Accession No. 070661. Study No. SRI Project LSC-7182.

1. Clearly identify the Half-Resolved ZR-3210 Technical as to its composition and purity.

2. Clearly identify ZTS-0017 as to its composition and purity.

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Eye Irritation Study - Rabbit. Accession No. 070100. Study No. SRI Project LSC-7182, Report No. 18.

1. A clear and total chemical identification of:

   a. ZPA - 1156
   b. ZPA - 1048
   c. ZR - 32 0 25

2. Submission of laboratory records containing the recorded results of the original eye observations, as well as, the method of data tabulation.

3. Method of eye examination.

Two Generation Rat Reproduction Study. Accession No. 070660. Study No. 322-039.

1. The Toxicology Branch does not disagree with the conclusion that the NOEL for the study is 20 ppm. However, we would ask the petitioner to submit a line of reasoning which separates those effects for the study which have led to a NOEL for the study, from those effects which have led to a NOEL for reproductive effects per se.

2. Identify the strain of rat used in this study.

90-Day Dietary Toxicity Study in Mice. Accession No. 070663. Study No. LBI Project No. 22088.

1. The protocol states that all gross lesions were to be examined microscopically. Why weren't the ovarian cysts which were observed grossly in the Group 3 and 4 females examined microscopically?

2. Did the animals give any indication of itching or scratching?

3. Were the lesions of sufficient size or character to affect the blood parameters?

Albin B. Kocialski, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

OPP:HED:TOX:A.KOCIALSKI:sb 10/6/82 X77395 #m17

002256

Subject: Acute Oral LD_{50} - Rat

Test Compound: Mavrik(R) 2E
Insecticide Acaricide Formulated from
Half-Resolved Fluvalinate Technical.

Sample Identification Code: Mavrik(R) 2E ZPA 1457

Accession No. 070664

Testing Facility: WIL Research Laboratories, Inc. Cincinnati, Ohio

Study Number: WIL - 80203

Responsible Professionals:

Lois Abbott - Technician

Linda Altringer - Technician

Arlene F. Kingery - Section Head

Dale Mayhew - Study Director

Testing Period: December 4, 1980 - December 24, 1980

Report Submitted to Sponsor: February 1981

Materials and Methods: Young adult male and female albino rats of the Sprague - Dawley CD Strain were obtained from Harlan Industries, Inc., Indianapolis, Indiana. Upon arrival, all animals were examined by a licensed staff veterinarian and quarantined. Rats were then sexed and housed in wire bottomed cages and allowed free access to Purina Certified Rodent Chow 5002(R) and tap water. Animals were then allowed to acclimate to laboratory conditions. On the last day of the acclimation period (i.e., the last day prior to initiation of exposure to the test article) five groups, each consisting of ten rats (5 males and 5 females), were selected for study using a computer-generated list of random numbers. Each rat was then weighed (animals were weighed on days -1, 0, 6 and 13) and ear punched. Food was withheld for a period of 24 hours prior to dosing. However free access to food was allowed one hour after dosing. The dose levels employed are tabulated below.

Group	Actual Dose Am't (ml/kg)	(Dose Level (g/kg)
1	1.0	0.1
2	0.5	0.5
3	1.0	1.0
4	2.5	2.5
5	5.0	5.0

The low dose group received a solution of test material and vehicle using distilled water (10% v/v) to provide an adequate dose amount for this group. The other four dose groups were dosed with the undiluted test material. A control group was not employed.

All animals were observed hourly for the first five hours post-dosing for gross signs of systemic toxicity and mortality. Animals were then observed twice daily thereafter for a period of 14 days. Terminal body weights were taken on those animals found dead. Animals surviving the 14 day period were sacrificed by carbon dioxide inhalation and a gross necropsy performed.

The acute oral LD_{50} was calculated using the method of Litchfield and Wilcoxon.

Results: Mean body weight gains appeared normal for survivors. Animals found dead showed a marked decrease in body weights. This reviewer notes here that dehydration of the carcass may be a factor as related to the length of time the animal was dead before being found and weighed.

Systemic signs were observed for all dose levels with signs occuring as early as one-half hour and lasting as late as five hours post-dosing.

All groups manifested inactivity, salivation, a small amount of dried red material around nose/eyes, loose feces and excreta stains.

Groups 2, 3, 4 and 5 manifested lethargy, ataxia, labored and slow respiration, lacrimation, intermittent scratching/digging at the cage and a white precipitate in the mouth at the time of dosing.

Group 3 manifested mastication, reddened extremities, and violent jumping with the head hitting the top of the cage. Some bodies were cool to the touch and stiff.

Group 5 manifested mastication, vocalization, glazed eyes and prostration.

MORTALITY

Group	Males	Females
1	0	0
2	1	0
3	1	2
4	4*	5
5	5	5

* The one animal that survived was not fasted before dosing. This animal was not included in the LD_{50} calculation.

Necropsy revealed no significant gross pathologic findings in Groups 1 and 2. Two females of Group 3 revealed hemorrhage of the hind stomach. All other animals showed negative gross pathological findings. All animals in Group 4 revealed a whitish intestinal content. The lone survivor was negative for gross pathology. All animals in Group 5 showed evidence of enteritis.

Acute Oral LD_{50} Calculation for Mavrik(R) 2E ZPA 1457

Males: 1109.0 mg/kg

Females: 1052.0 mg/kg

Combined: 1097.0 mg/kg (95% C.L. 726 to 1656 mg/kg; slope 1.60 with 95% C.L. 1.37 to 1.87).

Catagory of Toxicity: III

Classification: Core-Guideline

Reviewers Note: Males and female rats were observed intermittently "scratching at the cage" or "digging in the cage". This sign was recorded as a behavioral/CNS effect, and occurred as early as one-half hour and no later than 5 hours post dosing. The extracted data for this phenomenon is duplicated below.

MALES

Time (Hrs.) Dose (g/kg)	1/2	1	2	3	4	5
0.1						
0.5	38(s)					
1.0		37(d)	37(d)			
2.5	all(s)		all(s)			
5.0						

Legend: 38 = animal number
S = scratching
D = digging
all = all animals in group

FEMALES

Time (Hrs.) Dose (g/kg)	1/2	1	2	3	4	5
0.1						
0.5		173(s)230(s)				191(s)
1.0		all(s)(D)		all(s)(D)		
2.5						
5.0						

This observation is noted here in light of the scratching and lesions observed in subchronic rodent feeding studies as well as in the subchronic dog study (oral gavage). These signs appear to be dose-related and transient with time. The intensity of the effect was not reported. The effect was only reported as being present. The fact that no observations were reported above 2.5 g/kg for males and above 1.0g/kg for females does not necessarily exclude the presence of a dose response as the toxic effects seen at higher levels may well have masked the expression of the phenomenon which was observed at the lower dose levels. The presence of other mitigating factors was not excluded (e.g. mites). It should also be noted that a control group was not employed. This observation was not previously reported in any other acute oral (gavage) study to this reviewer's knowledge.

The following questions have been raised in the course of the review and are directed to the petitioner for a reponse.

1. What is the white precipitate a sign of in the mouth of the rats?

2. Would the cause of this sign affect the results of the study?

3. We ask the petitioner to address the signs "scratching and digging" in this study in light of the scratching phenomenon observed in other studies, as well as the appearance of skin lesions in other studies (i.e., rats and dogs).

002256

Subject: Acute Dermal LD_{50} - Rabbit

Test Compound: Mavrik(R) 2E Insecticide Acaricide Formulated from Half-Resolved Fluvalinate Technical

Sample Identification Code: Mavrik(R) 2E ZPA 1457

Accession No.: 070664

Testing Facility: WIL Research Laboratories, Inc.
Cincinnati, Ohio.

Study Number: WIL 80203

Responsible Professionals:

Lois Abbott - Technician

Linda Altringer - Technician

Arlene F. Kingery - Section Head

Dale Mayhew - Study Director

Fred W. Sigler - Pathologist

Testing Period: December 16, 1980 - December 29, 1980

Report Submitted to Sponsor: February 1981

Materials and Methods: Young adult male and female New Zealand strain white rabbits were received from J and J Research Farms of Hamilton, Onio. Upon arrival, all animals were inspected by a staff veterinarian. Each rabbit was sexed, weighed, eartagged and individually housed in wire-bottomed cages. Rabbits were quarantined and acclimated to laboratory conditions for 13 days prior to study initiation. Purina Certified Rabbit Chow 5322(R) and tap water were offered ad libitum.

The day prior to initiation of exposure to the test compound, three groups of 5 males and 5 females per group were formed. Body weights ranged from 1.90 to 2.85 kilograms. The backs of the animals were shaved with the shaved area constituting about 10% of the total body surface area. A 24 hour recovery period followed.

Three dose levels were applied.

Group	Actual Dose Am't (ml/kg)	(Dose Level mg/kg)
1 (vehicle control)*	2.10	2100
2	0.21	210
3	2.10	2100

*Distilled water.

Just prior to the application of the test material, the skin test site on all of the test and control animals was abraded with a 25-gauge hypodermic needle. The abrasions were made sufficiently deep to penetrate the stratum corneum but not to disturb the derma or to produce bleeding.

Each test site was immediately occluded with a layer of 4-ply gauze, two single layers thick. The trunk of each rabbit was wrapped with rubber latex dental dam and taped at the edges with 1 inch Micropore tape to form an airtight occlusive wrap. To prevent oral ingestion of the test material, each rabbit was maintained in a Newman harness for the 24 hour exposure period. At the end of the exposure period the harness and wrappings were removed and the residual test material wiped off. The test site was examined for local skin reactions for 14 consecutive days using the criteria of Draize. Animals were also observed for gross signs of systemic toxicity and mortality. Animals were also weighed on days 0, 6, and 13.

All animals were sacrificed by Euthanasia T-61 and a gross necropsy performed.

Results: Body weight changes were not significant. Systemic toxicity was not evident and no deaths occurred during the study. Loose feces and fecal stains, ocular and/or nasal discharges and lacrimation occurred in all dose groups and appeared not to be test compound related. Evaluation of local skin reactions were reported as follows:

Vehicle control group: Slight erythema during days 1-5 in 7/10 animals.

Dose level of 210 mg/kg: Erythema: Slight to moderate erythema was observed during days 1-10 for all animals. Edema: Slight edema was also observed during days 1-6 in all animals.

Eschar formation and skin necrosis was not observed. Scaling of the skin was observed in several animals of both sexes with slight to moderate severity.

Dose level of 2100 mg/kg: Erythema: Slight to moderate erythema was observed in all animals during days 1-13. Edema: Slight to severe edema was observed during days 1-13 in all animals.

Eschar formation and skin necrosis was not observed. Scaling and dried skin first occurred on day 4 and lasted until day 13 in 9/10 animals.

Slight to moderate atonia was observed on day 3 and lasted in 5/10 animal until day 13.

Necropsy and Pathology: No gross pathology was observed in the examination of the visceral and thoracic cavities. Examination of the exposure sites by the pathologist revealed positive histopathological findings in 3/10 animals of the high dose group.

Summary and Conclusion: Local skin reactions were related to dose level with the severity and the number of animals increasing as the dose was increased. Histopathologic evaluation of the exposure site revealed focal

Munro microabscesses in 3/10 (2 males and 1 female) animals in the high dose group consistent with an allergic response of the skin.

Acute dermal LD_{50} of Mavrik(R) 2E ZPA 1457 is greater than 2100 mg/kg of body weight in rabbits.

Toxicity Catagory: III

Classification: Core - Guideline

Note: The following reference was provided by Fred W. Sigler, D.V.M. Pathologist.

According to Lever, W. (Histopathology of the Skin, 2nd ed., J.B. Lippincott Co., Philadelphia, pp 66-75) Munro microabscesses are consistent with dermatitis-eczema, and inflammation of the skin, based on an allergic response of the skin to a variety of agents.

002256

Subject: Primary Skin Irritation Study in Albino Rabbits.

Test Compound: Mavrik(R) 2E Insecticide Acaricide Formulated from Half-Resolved Fluvalinate Technical.

Sample Identification Code: Mavrik(R) 2E ZPA 1457

Accession No.: 070665

Testing Facility: WIL Research Laboratories, Inc., Cincinnati, Ohio.

Study Number: WIL - 80203

Responsible Professionals:

Lois Abbot - Technician

Linda Altringer - Technician

Arlene F. Kingery - Section Head

Dale Mayhew - Study Director

Testing Period: December 2 - December 16, 1980

Report Submitted to Sponsor: February 1981

Materials and Methods: Young adult male and female New Zealand strain white rabbits were received from J and J Research Farms, Hamilton, Ohio. Upon arrival, all animals were inspected by a staff veterinarian. Each rabbit was sexed, weighed, eartagged and individually housed in wire bottom cages. Rabbits were quarantined and acclimated to laboratory conditions for 11 days prior to study initiation. Purina Certified Rabbit Chow 5322(R) and tap water were offered ad libitum.

The day prior to initiation of exposure to the test compound, 3 male and 3 female rabbits were selected and used for the study. The backs of the animals were shaved, with the shaved area constituting about 10% of the total body surface area. A 24 hour recovery period followed.

Exactly 0.5 mls. of the undiluted test material was applied to each of the 4 test sites on each rabbit. Two of the test sites were abraded with a 25 gauge hypodermic needle, two perpendicular to the other two. The abrasions were made sufficiently deep to penetrate the stratum corneum but not to disturb the derma or to produce bleeding. Abrasion sites were rotated counter clockwise. The other two skin sites remained intact.

Each test site was immediately occluded with a 1 x 1 inch square gauze patch, two single layers thick. The patches were secured in place by Micropore tape. The trunk of the rabbit was wrapped with rubber latex dental dam and held in place with Micropore tape to form an airtight occlusive wrap. Each animal was then placed in a Newmann harness for 24 hours to prevent oral ingestion.

The test material remained in contact with the skin for 24 hours. At the end of this period the harness, rubber wrappings, patches and tapes were removed and the residual material wiped off. The test site was examined for local skin reactions and scored and evaluated using the method of Draize. All surviving animals were observed for gross signs of systemic toxicity and mortality.

All animals were sacrificed by Euthanasia T-61 and a gross necropsy performed on the visceral and thoracic cavities.

To evaluate the average irritation present, the mean scores for erythema and edema of the intact test sites for the 24-hour and 72-hour grading period were added together and averaged to obtain the Intact Mean. Similarly the mean scores for erythema and edema of the abraded sites were added together and averaged to obtain the Abraded Mean. The total of the two means was then calculated to determine the primary irritation index.

Results: Body weights at termination appeared normal. No animal exhibited signs of systemic toxicity during the investigation. Positive irritation scores were observed at 72 hours. The sponsor was notified and in turn requested that the study continue until all scores were zero.

One animal was sacrificed on day 9. Gross pathologic findings in this animal did not appear to indicate a compound related effect. Necropsy of all other terminally sacrificed animals apparently were unremarkable.

Positive scores involved erythema and edema extending beyond the site at 24 and 72 hours and days 4 thru 9. The eschar formation occurred in only one animal and was attributed to damage of the dermal layer during physical removal of the tape. (Note: This explanation appears plausible since the acute dermal LD_{50} study in rabbits showed no eschar formation at 210 and 2100 mg/kg). The primary skin irritation score was determined to be 1.74.

Summary and Conclusion: When Mavrik 2E ZPA 1457 was administered at a dose of 0.5 ml per test site, four test sites per animal, to six albino rabbits (3M and 3F) no signs of systemic toxicity or compound related mortality were observed.

The primary skin irritation index of 1.74 classifies Mavrik(R) 2E ZPA 1457 as mildly irritating.

Category of Toxicity: III

Classification: Core Guideline

Subject: Skin Sensitization Study - Guinea Pig

Test Compound: Mavrik(R) 2E Insecticide Acaricide Formulated From Half-Resolved Fluvalinate Technical

Sample Identification: Mavrik 2E formulated from Run 23 R Half-Resolved Fluvalinate ZPA 1495.

Accession No: 070665

Testing Facility: Elars Bioresearch Laboratories, Inc., Fort Collins, Colorado.

Study No: Project No. 1649-E

Responsible Professionals:

Terry Hawett - Technician/Co-ordinator

Kris Hansen - Study Director

L. Steven Beck - Director of Toxicology

Testing Period: March 17 - April 22, 1981

Revised Report Submitted to Sponsor: June 1981

Materials and Methods: Young adult male albino guinea pigs were received from the Charles River Laboratories, Wilmington, Massachusetts. Animals were identified individually by ear notches and cage tags and were housed individually in stainless steel cages. Guinea pigs were allowed free access to Purina Guinea Pig Chow(R) and fresh water throughout the two-week acclimation period and during the study.

The study consisted of two parts:

o A dose titration to determine the irritation potential of the test material and,

o A subsequent skin sensitization study using the highest dose that was non-irritating.

A dose titration study was conducted on 2 animals to determine the degree of skin irritation. Undiluted test material plus 3 dose levels of diluted material were tested. The animals were clipped free of hair from shoulders to hips and half-way down either side of the thorax. The 4 patch sites were then depilated using Neet.(R) A dose of 0.5 ml of diluted (v/v 1:2; 1:4; and 1:8) or undiluted test material solution was applied to a one-inch square gauze sponge two layers thick and backed by an occlusive plastic wrap. Each animal received 4 patches held in place with conform elastic tape to prevent slippage of the patches. The animals were then returned to their cages for observation. The test material was kept in contact with the skin for six hours and then removed. The test sites were then scored using the system of

Draize for erythema and edema. No irritation was observed on either guinea pig.

A skin sensitization study was conducted using the undiluted test material as this was the highest dose that was non-irritating.

One patch containing a dose of 0.5 ml of undiluted material was applied to the depilated skin of each of ten guinea pigs which were left in place for six hours and then removed. Twenty-four hours after application the test sites were independently scored by 2 technicians for erythema and edema. The procedure was followed once a week for a period of 3 weeks (Ref. Ritz, Harry L., and Edwin V. Buchler, Planning Conduct and Interpretation of Guinea Pig Sensitization Patch Test. Current Concepts in Cutaneous Toxicity. pp. 25-40. 1980). The test sites were rotated on the backs of the test animals as judged necessary to reduce irritation. The same two technicians conducted all the scoring. Two weeks after the last application of the test material the guinea pigs received a single challenge treatment.

A positive control group consisting of ten guinea pigs was also employed according to the above procedure. A 0.05 percent (w/v) dilution of chlorodinitrobenzene in ethanol was used as the positive control material.

A challenge control group of ten animals was shaved and depilated with Neet(R) 24 hours prior to the challenge treatment and dosed with the test material in the same manner as described above at the time of the challenge treatment.

(Note: The challenge control group simulates an initial exposure to the test material so any response in the test group at challenge above that in the challenge control group is due to the sensitization reaction).

Sensitization was deemed to occur if the majority of animals in the group exhibited elevated scores at challenge compared with initial treatment.

Results: Skin reactions scored independently by two technicians were comparable.

Scores from the positive control group and the test group increased from treatments 1 to 3. However, the challenge dose to the test group had significantly lower scores than those recorded for its own previous treatments numbered 1, 2 and 3. The challenge dose in the positive control group produced results comparable to those found in its own immediately previous two treatments.

The challenge dose results in the test group when compared to the results of its own first treatment were similar numerically. The challenge dose results in the positive control group were substantially higher when compared to its own first treatment results and higher than those results recorded for the challenge dose in the test group.

The challenge control group produced numerical results which were less than those noted in the positive control group but which were greater than those observed in the test group.

Additionally, of the animals in che test group, two had slight erythema at challenge but these same animals showed no response at the end of the first treatment whereas the remaining animals had generally identical or generally less erythcma at challenge than after the first treatment. Edema was comparable at both treatment 1 and challenge.

In the positive control group 8/10 guinea pigs had higher scores for erythema and 4/10 had higher scores for edema after challenge than after the first treatment. Fewer animals in the test group had erythema at challenge than the challenge control. Elevated scores in the majority of positive control animals after challenge indicate that sensitization occurred. Most test animals had no increase in erythema after challenge compared with scores generated after only one treatment.

Conclusions: Mavrik(R) 2E formulated from half-resolved fluvalinate is non-sensitizing under test conditions.

Category of Toxicity - Non-Sensitizing

Classification - Core - Guideline.

Subject: Primary Eye Irritation Study in Albino Rabbits.

Test Compound: Mavrik(R) 2E Insecticide Acaricide Formulated from Half Resolved Fluvalinate Technical

Sample Identification Code: Mavrik 2E ZPA 1457

Accession No: 070665

Testing Facility: WIL Research Laboratories, Inc., Cincinnati, Ohio.

Study No.: WIL - 80 .03

Responsible Professionals:

Lois Abbot - Technician

Linda Altringer - Technician

Arlene Kingery - Section Head

Dale Mayhew - Study Director

Testing Period: December 2, - December 31, 1980

Report Submitted to Sponsor: February 1981

Materials and Methods: Young adult male and female New Zealand strain white rabbits were received from J and J Research Farms, Hamilton, Ohio. Upon arrival all animals were inspected by a staff veterinarian. Each rabbit was sexed, weighed, eartagged and individually housed in wire bottom cages. Rabbits were quarantined and acclimated to laboratory conditions for 11 days prior to study initiation. Purina Certified Rabbit Chow 5322(R) and tap water were offered ad libitum.

Prior to initiation of exposure to the test article, 5 male and 4 females were selected for use. Approximately 24 hours prior to application of the test material, both eyes of each animal in the test group were examined using fluorescein dye procedures. All test animals were without defects or irritation.

Exactly 0.1 ml of the undiluted test material was instilled with a sterile syringe in the right eye of each animal by gently pulling the lower lid (conjunctival cul-de-sac) away from the eyeball to form a cup into which the test substance was dropped. Following instillation of the test material, the eyelids were held closed for 1 second and then released. Thirty seconds after instillation of the test material, the treated eyes of 3 of the rabbits were gently held open and rinsed with two 60cc syringes filled with lukewarm distilled water for one minute.

All animals were observed for gross signs of systemic toxicity and mortality for a total of 28 days.

Grading for irritation and injury were made at 24, 48 and 72 hours and also at 4, 7, 14 and 21 days. Two male animals, one of which had the eye rinsed and one which did not, were also graded on days 24 and 28. The standard scoring system of Draize was used in grading for irritancy and injury. Fluorescein examinations were also used to facilitate the evaluation of potential corneal damage.

All animals were weighed at termination, sacrificed, using Euthanasia T-61 and had a gross necropsy performed on the visceral and thoracic cavities.

Results: Body weight gains at termination appeared normal. No animals exhibited signs of systemic toxicity and no deaths occurred during the experiment. Necropsy of the visceral and thoracic cavities of the test animals revealed no significant findings.

Positive scores noted involved the cornea, iris and conjunctiva lasting up through the 21 day period in 3/9 (2 animals rinsed eye, 1 animal not rinsed) of the animals. The sponsor requested that the 3 animals with positive scores be continued on for another seven days. One animal was inadvertently sacrificed with the other six. The two remaining animals were evaluated and scored at 24 and 28 days. The animal which did not have its eye rinsed demonstrated a complete recovery by day 28; the other animal showed corneal involvement and chemosis.

Additional observations noted at the different evaluation and scoring intervals were: white material present immediately after dosing (9/9), thick white material partially covering the corneal surface and imbedded in the lower and upper conjunctiva (7/9), thick yellow-white discharge (9/9), puckering of lower or upper lid (6/9), severe hair loss around eye (1/9), and pannus (2/9), irregularity of the corneal surface (9/9).

Mean eye irritation scores for the first 4 days were generally similar for all animals ranging between 41 and 47 with values peaking on day 4 (mean score on day 4 for those eyes not rinsed was 44.5 compared to a score 47.5 for those eyes which were rinsed). The 4-day interval provided the highest mean score and was therefore used as the primary irritation index. It was also noted that the mean scores for those animals whose eyes were rinsed were slightly higher at each interval after 72 hours when compared to the not-rinsed group.

Summary and Conclusion: The primary eye irritation index of 44.5 for eyes not rinsed and 47.5 for eyes rinsed at day 4 and a mean seventh day score of greater than 20 classify Mavrik(R) 2E ZPA 1457 as severely irritating. Instillation of water slightly increased mean scores after 72 hours but did not change the classification.

Corneal opacity persisted after 7 days.

Category of Toxicity: I

Classification: Core-Guideline.

Subject: Acute Oral LD50 - Rat. Final Report
Acute Oral LD50 - Mouse. Final Report

Test Compound: Half-Resolved Fluvalinate Technical; ZTS-0017 a new formulation of ZR-3210 Technical.

Accession No: 070661

Testing Facility: SRI International

Study Number: SRI Project LSC-7182

Responsible Professionals:

Carol J. Rushbrook - Toxicologist
Ted A. Jorgenson - Director, Mammalian Toxicology Department
David C. L. Jones - Direcctor Toxicology Laboratory
W. A. Skinner - Executive Director, Life Sciences Division

Testing Period: October-November, 1980 (best estimate)

Report Submitted to Sponsor: November, 1980

Purity of Test Material: Not given.

Batch or Lot Number: 1080022

Specific Gravity: 1.23 g/ml

Stability: Previous studies have shown the compound to be stable under test conditions.

Acute Oral LD50 Rats

Materials and Methods:

Young-adult Sprague-Dawley derived rats of both sexes were purchased from Simonsen Laboratories, Inc., Gilroy, California. The animals were held in an air-conditioned animal room for 6-10 days of acclimation before treatment. They were housed five per cage in polycarbonate shoe box cages with hardwood chip bedding; food (Purina Lab Chow®) and water (UV-purified) were available ad libitum. Groups of 10 males and 10 females each, weighing between 80 and 100 grams, were fasted overnite and given ZTS-0017 at levels of 56, 100, 178, 316, 562 and 1000 mg/kg. The compound was administered as a solution in corn oil at a constant volume of 1.0 ml of solution per 100 grams of body weight. All animals were observed closely immediately after treatment and several times later that day. Daily observations

for physiological and behavioral responses and mortality were continued for 14 days after treatment. Times of death were recorded. Body weights were taken initially and then weekly during the 2-week observation period. All animals that died during the study were necropsied for evidence of gross pathological changes. Survivors were sacrificed and necropsied at the end of the observation period. No tissue specimens were retained. The LD50 value was calculated by the methods of C. S. Weil (Biometrics, September 1952, p. 249).

Results:

Most deaths occurred within the first day of treatment and followed depression, moderate to profuse salivation and a humped appearance. Rats given 562 or 1000 mg/kg also had moderate or marked ataxia. Rats that did not die until the second or third day had diarrhea and appeared prostrated. Clinical signs were generally similar in surviving rats, with salivation, ataxia, depression and diarrhea being more pronounced at higher dose levels. All survivors appeared normal within 3 to 6 days after treatment and weight gains were steady through out the two week observation period. Gross necropsy of rats that died showed that one-fourth had moderately hemorrhaged lungs. No other gross abnormalities were found in either decedents or survivors.

Conclusion:

The acute oral LD50 and 95% C.L. of ZTS-0017 when administered to males was calculated to be 282 (218-365) mg/kg.

The acute oral LD50 and 95% C.L. for females was 261 (194-352) mg/kg.

Classification: Core-guideline Supplementary

Category of Toxicity is: II

Acute Oral LD50 - Mice

Materials and Methods:

Young-adult ICR/SIM mice of both sexes were obtained from Simonsen Laboratories Inc., Gilory, California. The quarantine and husbandry procedures were the same as for the rats. Fasted groups of 10 male and 10 females were treated with 80, 126, 200, 317, 502 or 796 mg/kg. Body weights for both sexes ranged from 18-26 grams. The administration of compound, observations, body weight recordings and necropsies were conducted as described above for rats.

The LD50 values were calculated using the Chi-square method of J. T. Litchfield and F. Wilcoxon.

Results:

Except for one female (126 mg/kg dose group) that died after one week, all mice that died did so within 20 hours of treatment. Mice in all dose groups exhibited salivation, ataxia and depression. Survivors were depressed and most also had a humped appearance on the day following dosing. Those survivors in the 80, 126, and 200 mg/kg groups appeared normal by day 3; the one female survivor in the 317 mg/kg group took one week to recover. Mice that survived gained weight throughout the observation period. Necropsy of decedents revealed no gross abnormalities with the exception of those attributable to poor technique (i.e. esophageal or stomach punctures). Those animals were not used in calculating the LD50 values. At the end of the two week observation period gross necropsy revealed a 57% incidence of slightly pale or mottled kidneys; no other abnormalities were noted.

Conclusion:

AOLD50 - males - 156 mg/kg 95% C.L. (123-198)
- females - 222 mg/kg 95% C.L. (135-364)

Classification: Supplementary. May be raised to Core-guideline pending resolution of questions 1 and 2.

Category of Toxicity is: II

The following questions havve arisen in the course of the review.

1. Clearly identify the Half-Resolved ZR-3210 Technical as to its composition and purity.

2. Clearly identify ZTS-0017 as to its composition and purity.

#ml4

Subject: Acute Oral LD50 - Rat (Second Test) Final Report

Test Compound: Half-Resolved Fluvalinate Technical; (Half-Resolved ZR-3210 Technical)

Accession No: 070661

Testing Facility: Elars Bioresearch Laboratories
Fort Collins, Colorado

Study No: Project No. 1654-D

Responsible Professionals:

Terry A. Hewett - Toxicology Technician
Kris L. Hansen - Acute Toxicology Study Director
L. Steven Beck - Director of Toxicology

Testing Period: April 13 thru April 27, 1981

Report Submitted to Sponsor: June 1981

Purity of Test Material: 93.1%

Batch or Lot Number: Run 23, Anal. No. 0281028

Stability: No stability data were provided. However, it is reasonable to assume that the compound is stable as its stability has been shown in other studies.

Material and Methods:

Healthy male and female Sprague-Dawley albino rats were obtained from Taconic Farms, Inc., Germantown, New York. The animals were randomized upon receipt, ear tagged, and housed individually in suspended cages. The animals were allowed free access to fresh water and Purina Rodent Chow®. A three week period of acclimation was observed. Males weighed 250-350 grams and females weighed 200-250 grams at the initiation of the study. Five test groups, each consisting of 5 males and 5 females per group were administered the following oral dosages of compound in a uniform volume of 10 ml/kg of corn oil; they were 50, 144, 250, 350 and 550 mg/kg of compound per body weight. A control group consisting of 5 males and 5 females was administered Mazola® corn oil only. Rats were fasted for approximately 16 hours prior to dosing. On the day of dosing rats were weighed and doses calculated. All animals were returned to their cages post-dosing and were observed for toxic signs or mortality several times during the day. The rats were observed twice daily for the duration of the 14 day study interval. Body weights were measured and recorded on the day of dosing (day 0), day 7 and at termination (day 14). All animals dying intercurrently were subjected to a gross necropsy. All surviving rats were killed and necropsied on day 14 of the study.

Results:

Animals receiving the test compound exhibited salivation, ocular discharge, diarrhea, lethargy and ataxia. The onset of these signs occurred approximately 1-2 hours after dosing. The severity and persistence of the signs increased with increased dosage. All signs subsided in all dose groups by day 7. Vehicle control rats appeared normal after dosing and throughout the 14 day observation period. Body weight gains appeared to be generally comparable between groups. Gross necropsy of rats that died on study revealed heavy salivation and diarrhea stains, stomach and intestine bloated with gas and several with fluid in the intestine. One animal had a congested liver and spleen. Tissues in 5 of 7 rats that died on study were autolyzed at necropsy. Necrospy of test rats that survived the 14 days on study revealed a thickening of the glandular region of the stomach in one low dose female animal; all other animals appeared normal. All vehicle control rats appeared normal at necropsy. No animals died in the two low dose groups whereas a 20% mortality was observed in each of the next two higher dose levels. Animals receiving the highest dose produced a mortality of 30%.

Conclusion:

Half-resolved ZR-3210 Technical, when administered by gavage to adult rats, was determined to have an oral median lethal dose of greater than 550 mg/kg under the conditions of the study.

Classification: Core-Guideline

Toxicity Category: III

#ml4

Subject: Acute Oral Screening (Range Finding) Toxicity Study in Rabbits. Final Report.

Test Compound: Racemic and Half-Resolved ZR-3210 Technical Fluvalinate

Accession No: 070661

Testing Facility: Hazleton Laboratories America, Inc.

Study Number: Project No. 777-134

Responsible Professionals:

Julie A. Ralph - Technical Writer
Gary W. Wolfe - Project Co-ordinator, Toxicology Department

Testing Period: First Set: (Racemic ZR-3210) August 26 - September 17, 1980

Second Set: (Half-Resolved ZR-3210) March 17 - April 10, 1981

Report Submitted to Sponsor: Revised Final Report August 17, 1981

Purity of Test Material with Batch or Lot Number:

Racemic ZR-3210 Technical - Lot No. ZCD-7R, Anal. No. 0280092; 93.0% pure

Half-Resolved ZR-3210 Technical - Run 23, Anal. No. 0281028; 93.1% pure

Stability: The compound has previously been shown to be stable under test conditions.

Materials and Methods:

New Zealand White female rabbits were obtained from Dutchland Laboratory Animals, Inc.,Denver, Pennsylvania. Two rabbits were selected from a larger pool of animals which had been randomized and these two animals comprised Set 1 (i.e. the group to receive the racemic mixture). Three additional rabbits were selected from a second pool by the same randomization process and comprised Set 2 (i.e. the group to receive the half-resolved mixture). The first set of rabbits was acclimated to laboratory conditions for approximately three months prior to initiation of treatment. The second set of rabbits was acclimated to laboratory conditions for approximately 5 weeks prior to the initiation of treatment. The rabbits were identified by individual animal numbers and housed individually in elevated metal cages marked with the

corresponding animal numbers. Commercial rabbit ration (Purina Lab Rabbit Chow®) and tap water (automated watering system) were available ad libitum. Dosing solutions were prepared on a weight per weight basis and adjusted to 100 percent of active ingredient. Corn oil (Duke's® Pure Corn Oil, C. F. Sauer Company, Richmond, Virginia) was added to each solution to achieve the desired concentration. All animals were dosed by the oral route as noted in the table below. Control groups were not used in either experiment.

Set 1

Racemic ZR-3210 Technical Fluvalinate

Group	No. of Animals Females	Dosage Level mg/kg	Dosing Volume ml/kg
1	1	1000	5.0
2	1	2000	5.0

Set 2

Half-Resolved ZR-3210 Technical Fluvalinate

Group	No. of Animals Females	Dosage Level mg/kg	Dosing Volume ml/kg
1	1	500	2.0
2	1	1000	2.0
3	1	2000*	2.5

* Thirteen days after the initial dose, this rabbit was dosed at 2000 mg/kg for five consecutive days.

All of the rabbits were observed for mortality and signs of toxic and pharmacologic effects immediately after dosing and twice daily thereafter for fourteen (14) consecutive days. One rabbit in the second set (half-resolved group) dosed at 2000 mg/kg of body weight was observed daily from days 15-22 in order to carry out subsequent dosing. Individual body weights were recorded prior to treatment in all rabbits and at termination in the first set (racemic group) of rabbits. Individual body weights were also recorded on day 13 and 18 in the same rabbit that received subsequent dosages. Terminal body weights were inadvertently not recorded for Groups 1 and 2 of Set 2. All rabbits were sacrificed with T-61® Euthanasia Solution (Taylor Pharmacal Company, Decatur, Illinois).

Results: Set 1 (Racemic Mixture):

No deaths occurred. The rabbit dosed at 1000 mg/kg remained normal except for urine stains on day 2 and anorexia on days 3-5. The rabbit dosed at 2000 mg/kg remained normal throughout the study. The animal dosed at 1000 mg/kg lost 105 grams (-2.6%) of body weight from initiation (4110 g) to termination (4005 g) of the study, while the animal dosed at 2000 mg/kg lost 150 grams (-4.4%) of body weight from initiation (3430 g) to termination (3280 g) of the study.

Set 2 (Half-Resolved):

No deaths occurred and all animals remained normal except for anorexia reported on the following days:

Dose (mg/kg)	Days
500	2, 3, 4, 12 and 14
1000	2
2000*	2, 3, 16, 17, 19 and 20

* Thirteen days after the initial dose, this rabbit was dosed at 2000 mg/kg for 5 consecutive days.

Terminal body weights were inadvertently not recorded for these animals. The animal dosed at 2000 mg/kg and then 2000 mg/kg for 5 consecutive days weighed 3490 grams on day 1 amd 3400 gms on day 13 and finally 3080 grams at the completion of dosing (day 18). This loss represented a 9.4% loss in body weight over the 5 day period.

No observable gross pathology was noted in any of the rabbits.

Conclusion:

The test results strongly suggest an AOLD50 of greater than 2000 mg/kg in rabbits for both the racemic and half-resolved mixtures. This would place the product in at least Category III for oral toxicity.

Classification: Supplementary.

Subject: Eye Irritation Study (Rabbit) Final Report

Test Compound: Mavrik® 2E (Diluted for Use)

Accession Number: 070100

Testing Facility: SRI International

Study Number: SRI Project LSC-7182, Report No. 18

Responsible Professionals:

Carol J. Rushbrook - Biologist
Ted A Jorgenson - Manager, Mammalian Toxicology Program
David C. L. Jones - Director, Toxicology Laboratory
W. A. Skinner - Executive Director, Life Science Division

Testing Period: December 1979 (Best Estimate)

Report Submitted to Sponsor: December 1979

Background

This report describes the results of the rabbit eye irritation study of a 10% aqueous solution of ZR-3210 2E. Zoecon supplied 10 ml of the compound, which was identified as formulation ZPA 1048 (a 10% dilution of ZPA 1156).

Materials and Methods

Nine young-adult male New Zealand white rabbits, weighing 2 to 3 kg each, were purchased from Elkhorn Rabbitry, Watsonville, California. After several days of acclimation in an air-conditioned animal room, the rabbits were examined to ensure that there was no previous injury to the eyes. Then the test compound (0.1 ml) was instilled into the right eye of each rabbit; the left eye served as the reference control. In three rabbits, the compound was washed from the eye 30 seconds after instillation; in the remaining six, the compound was not washed from the eye. Each eye was graded for lesions 24, 48, and 72 hours after treatment. Additional readings were made on days 7 and 14. The grading system was taken from the Draize test.

Results

The 30-second wash group exhibited no corneal opacity during the study. A Grade 1 iris reaction was noted for the three rabbits for the 24- and 48-hour readings. By 72 hours after instillation, low scores represented minor conjunctival inflammation. By day 7, all signs of irritation had cleared from the eyes of the three rabbits.

Two rabbits in the no-wash group had corneal opacity, but no severe reactions, such as pannus, were noted. Only one rabbit had corneal opacity that lasted more than 1 day; a small area of translucence was present for the first 72 hours, which was less dense by day 7 and was clear on day 14. Iritis was seen in 5 of the 6 rabbits at different times during the first 3 days; only 1 rabbit still had it on day 7; no iritis was evident by day 14. Except for 1 rabbit with slight conjunctival redness, all eyes were normal on day 14 after treatment. The rabbits were sacrificed at that time.

Conclusion

The 10% aqueous solution of ZR-3210 2E is considered an irritant to the rabbit eye.

Category of Toxicity: Category II

Classification: Supplementary

Comments - The registrant must supply the following additional information before an upgrading of this study can be considered.

1. A clear and total chemical identification of:

   (i) ZPA - 1156
   (ii) ZPA - 1048
   (iii) ZR - 3210 2E

2. Submission of laboratory records containing the recorded results of the original eye observations, as well as, the method of data tabulation.

3. Method of eye examination.

Subject: Subchronic Rabbit Dermal Range Finding Study. Final Report.

Test Compound: Half-Resolved Fluvalinate Technical; ZR-3210 Technical; Mavrik® Technical

Accession No: 070664

Testing Facility: Elars Bioresearch Laboratories, Inc.

Project No: 1648-P

Responsible Professionals:

Terry A. Hewett - Toxicology Technician/Co-ordinator
Kris L. Hansen - Study Director
L. Steven Beck - Director of Toxicology

Testing Period: March 2 - March 23, 1981

Report Submitted to Sponsor: October 1981

Purity of Test Material: 93.1%

Batch and Lot No: Run 23R, Analytical No. 0281037; ZTS-0029.

Stability: The compound has been shown to be stable under ordinary conditions of laboratory testing, and storage.

Materials and Methods:

Four male and four female New Zealand White rabbits were obtained from the L.I.T. Rabbitry, Whitehall, Monatana. Rabbits were randomized upon receipt, placed in individual cages and identified with a unique ear tag. A two week acclimation period followed. During the second week of acclimation, all rabbits were introduced to Elizabethan collars for the purpose of minimizing any oral ingestion of the applied test material. Throughout the 2 week acclimation period and during the study animals were given free access to Purina Rabbit Chow® and fresh water. The fresh water was supplemented with adequate levels of vitamins B_1, B_2, and pantothenic acid. Vitamin B_6 was provided ad libitum for four days prior to study initiation.

Twenty-four hours before the application of the test material, the rabbits were shaved free of hair (10% of total body surface), weighed and returned to their cages.

Prior to application of the test material the exposure sites (one near the shoulder and one near the lower back) were abraded. Four epidermal incisions were made with a needle -- two parallel to the rabbits long axis and two perpendicular to the first abrasions. The abrasions, minor incisions through the stratum corneum, were not sufficiently deep to disturb the dermis or to produce bleeding.

Two rabbits were used as controls and the remaining animals dosed at a level of 2.0 g/kg/day.

The test material was applied to 3" x 3" gauze sponges backed with plastic wrap. The sponges and plastic wrap were then secured with a porous adhesive tape and the entire trunk wrapped with elastic. Animals were then returned to their cages. The bandaging and any residual material were removed at the end of six hours. The residual test material was removed using Johnson's Baby Shampoo®. The test site was then rinsed with water.

The above procedure was repeated five days per week for one week. The application site was, however, rotated between the two test sites on a daily basis.

Animals were observed at least once each morning and late afternoon for pharmacotoxic signs and general health. Skin at the test site was scored for irritation 24 hours after dosing and 24 hours after unwrapping using the system of Draize (1959). Animal body weights were taken on day 0, 4 and at termination of the study. All animals were sacrificed on day 7 with T-61® and subjected to gross necropsy.

Results:

Rabbits in both test and control groups appeared normal throughout the 7 day observation period.

No dermal irritation was in evidence at the test site in either group.

Some slight weight loss was observed in the test group in contrast to control animals which showed a slight weight gain for the same period.

Two rabbits in the test group had kidneys with pale cortices. Another rabbit in the test group had a liver mottled with white hard areas. In the control group one animal had an abscess on the right salivary gland. No other abnormalities were observed in either group.

Conclusion:

The test material, ZR-3210 Technical Half-Resolved, caused no dermal irritation, signs of systemic toxicity or mortality under the conditions of the test. Some slight weight loss was observed.

Classification: Supplementary.

#m15

Subject: Subchronic 21-Day Dermal Toxicity Study - Rabbits. Final Report

Test Compound: Half-Resolved Fluvalinate Technical
Mavrik® Technical
ZR-3210 Technical

Accession No: 070664

Testing Facility: Elars Bioresearch Labs.

Project No: 1675-F

Responsible Professionals:

Terry Hewett - Study Co-ordinator
Kris Hansen - Study Director
L. Steven Beck -Director of Toxicology
Donald N. Kitchen, D.V.M. - Pathologist

Testing Period: June 23, 1981 - July 17, 1981

Report Submitted to Sponsor: October 1981

Purity of Test Material: 93.1%

Batch and/or Lot No: Run 23R Analytical No. 0281037; ZTS-0029

Stability: The test compound was shown to be stable under ordinary test conditions.

Materials and Methods

Ninety young adult New Zealand White rabbits, evenly divided by sex, were obtained from the Elkhorn Rabbitry, Watsonville, California. Animals were randomized upon receipt and identified by a unique ear and cage tag. Rabbits were housed individually in suspended galvanized steel rabbit cages in a room controlled for temperature, humidity and a 12 hour light/dark cycle. Rabbits were acclimated for 3 weeks prior to study initiation. All animals had free access to Purina Rabbit Chow® and tap water. Water analysis for pesticides, organics, heavy metals and bacteria are conducted quarterly by the laboratory and the results are available upon request.

Culling of animals occurred at the end of the acclimation period and 80 healthy rabbits were randomly assigned to 4 treatment groups. The treatment groups were as follows:

Groups	Dose (mg/kg)
1 (control)	0
2	100
3	500
4	2000

Each group consisted of 10 does and 10 bucks and doses were adjusted to 100% of the active ingredient.

Rabbits were further divided into four replicates as follows:

			------Number of Rabbits--------			
Replicate	Sex	Test Site	Group I	Group II	Group III	Group IV
1	Male	Intact	1	2	1	2
		Abraded	1	1	1	1
	Female	Intact	1	1	1	1
		Abraded	2	1	2	1
2	Male	Intact	1	1	1	1
		Abraded	1	2	1	2
	Female	Intact	2	1	2	1
		Abraded	1	1	1	1
3	Male	Intact	2	1	2	1
		Abraded	1	1	1	1
	Female	Intact	1	1	1	1
		Abraded	1	2	1	2
4	Male	Intact	1	1	1	1
		Abraded	2	1	2	1
	Female	Intact	1	2	1	2
		Abraded	1	1	1	1

Twenty-four hours prior to the first treatment of each week, the dorsal aspect of each rabbit was clipped free of hair and consisted of not less than 10% of the total body surface. Animals were then weighed and returned to their cages for testing the following day. The 24 hour recovery period allowed for healing of any micropscopic abrasions possibly induced during the shearing process.

On the first treatment day of each week, prior to dosing, the skin of 5 rabbits (Note: 5 is really an average) of each sex per dose group was abraded. Four epidermal incisions were made with a needle - two parallel to the animals long axis and two perpendicular to the first abrasions. The abrasions were not sufficiently deep to disturb the dermis or to produce bleeding. Skin at the test site of the remaining 10 test rabbits (5 males and 5 females) in each group was left intact.

The appropriate dose (ml) for each rabbit was calculated by multiplying the dosage (g/kg) times the weight of the rabbit (kg) and dividing by the percent of active ingredient and specific gravity (1.24 g/ml) of the test material.

The test material was then spread on a 4 X 4 inch gauze backed with occlusive plastic wrap. The gauze and plastic wrap were taped to the clipped area of the rabbit's dorsal aspects with Dermilite II, a surgically non-allergic adhesive tape. The entire trunk was then wrapped with an elastic bandage to prevent slippage of the patch. Animals were then collared with Elizabethan collars and returned to their cages.

The material remained in contact with the skin for 6 hours per day. At the end of 6 hours, collars and bandaging were removed, and the residual test material removed using Johnson's Baby Shampoo® followed by a water rinse.

Control animals were treated in a similar manner, including shampoo and collar, but they did not receive the test material.

Observations and Measurements:

All rabbits were observed twice daily for general appearance, behavior and local reactions and signs of toxic or pharmacologic effects. Daily observations were recorded. Twenty-four hours after each wrapping, rabbits were scored for erythema and edema according to the method of Draize. Two technicians observed and evaluated skin reactions each day of scoring. Two of three technicians scored the dermal irritation throughout the study.

Each rabbit was weighed twice weekly and prior to termination. Mean biweekly weight gains were computed and compared for statistically significant differences among groups. Doses were calculated based on the first weighing each week.

Feed consumption was measured throughout the study. Group means for average daily feed consumption were compared for significant differences.

Blood was collected from 10 randomly selected rabbits (5 males, 5 females) per group one day prior to initiation of dosing and on day 22 of study. Rabbits were fasted for at least 12 hours prior to collecting blood samples.

Hematology and clinical chemistry determinations were made and group mean determinations were calculated and compared statistically.

Hematology parameters determined were:

- Total and differential leukocyte counts
- Erythrocyte count
- Hematocrit
- Hemoglobin
- Platelet count
- Reticulocyte count (if signs of anemia were present)

Clinical chemistry parameters determined were:

- Total protein
- Albumin
- Globulin
- Serum alkaline phosphatase (SAP)
- Serum glutamic-oxaloacetic transaminase (SGOT)
- Serum glutamic-pyruvic transaminase (SGPT)
- Serum lactic dehydrogenase (LDH)
- Blood glucose
- Blood urea nitrogen (BUN)
- Total bilirubin (if abnormal, direct bilirubin was determined)
- Total cholesterol
- Calcium
- Potassium
- Sodium

Blood samples were also collected from animals that died during the study.

A gross necropsy was performed on all rabbits that died on study as well as rabbits surviving to termination. Each rabbit was sacrificed with T-61® injected I.V. prior to necropsy.

Gross necropsy included examination of the following:

Carcass
External surface
All orifices
Cranial cavity
External and cut surfaces of the brain and spinal cord
Thoracic, abdominal, pelvic cavities and their viscera
Cervical tissues, and organs.

The following tissues were extirpated and weighed:

Liver
Kidney
Heart
Brain (with stem)
Gonads
Adrenal
Thyroid (plus parathyroid)
Pituitaary gland

Organ/body weight ratios and organ/brain weight ratios were calculated. Additionally, mean organ weights and organ weight ratios were calculated for each group and compared statistically.

Selected tissues were removed from each animal and fixed in 10% buffered formalin solution (testes in Bouins solution). They were:

heart
liver
adrenals
thyroid/parathyroid
pituitary
skin (treated/untreated)
brain stem
cerebellum
cerebrum
sciatic nerve
spinal cord (cervical, lumbar, thoracic)
epididymis
testes
ovaries
kidney
cecum
colon
eye
Harderian gland
jejunum
spleen
stomach

Post-mortem specimens were processed in the histology laboratory at Elars; however, the fixed tissues (hematoxylin and eosin stain) were submitted to Westpath Labs Inc. (Fort Collins, Colorado) for evaluation. The following tissues and organs were subjected to histopathologic examination:

Multiple sections of treated and untreated skin
All gross lesions (including some apparently normal contiguous tissue)
Liver
Kidney
Brain (3 levels - from cerebellum, cerebrum, pons)
Spinal cord (cervical, lumbar, thoracic)
Pituitary
Heart
Thyroid with parathyroid
Adrenals
Gonads
Distal sciatic nerve

Statistical Analysis:

Body weight, feed consumption, hematology, clinical chemistry and absolute organ weight data were recorded on computer for statistical analysis.

An analysis of variance was performed on each listed parameter. Whenever a probability value of less than 0.05 was found, a Tukey's HSD procedure was performed in order to detect statistically significant differences among groups.

Results

Daily observations:

No compound related deaths were noted.

No compound related observations were observed for the following parameters, depression, diarrhea, soft stool, dehydration, bloating, lacrimation, loss of appetite, skin irritation (other than the treatment site) and mortality.

Erythema - Abraded Skin

Males:

Examination of the submitted data indicated that a well defined erythema (Draize Score = 2) was observed in treated animals when compared to controls for all dose levels. A dose-response was not however evident and dermal irritation did not increase with time. All groups (including controls) manifested a Draize Score equal to 1.

Females:

Examination of the submitted data indicated that a well defined erythema (Draize Score = 2) was observed in treated animals when compared to controls for dose levels of 500 mg/kg and 2000 mg/kg. The data also suggested the presence of a barely perceptible erythema at 100 mg/kg. One female showed a severe erythema (Draize Score = 4) during the entire second week. This sign was not evident during the third week of observation.

Erythema - Intact Skin

Males:

A compound related effect was in evidence at all three dose levels, and was manifested by a well defined erythema (Draize Score = 2) with a shortened period of onset and an extended period of recovery with increased dose.

Females:

Females mainifested a well defined erythema (Draize Score = 2) with increased dose. The response was generally observed during the second and third week.

Edema - Abraded Skin

Males:

A barely perceptible edema was noted in treated groups.

Females:

A barely perceptible to a slight edema was reported at 500 and 2000 mg/kg.

Edema - Intact Skin

Males:

A barely perceptible edema was observed at all doses. The responses were however inconsistent with regard to time and dose.

Females:

Results which were suggestive of a barely perceptible edema at all dose levels were in evidence. However, results were not log-dose responsive, onset was not abreviated with increased dose and recovery was not prolonged with increased dose.

It was also reported that skin sores caused from rabbits biting themselves were observed in 8 animals. Sores were located adjacent to the test site on three animals, at the test site on one animal, and at a non-treated site on four animals. Collars were allowed to remain on 5 rabbits after unwrapping to prevent additional biting and to allow the skin to heal. These results are reproduced in tabular form below.

Animal Number	Dosage (mg/kg)	Location of Sore	Duration	Collared (Days of Study)
95	0	Edge of test site	Week 1	---
152	100	Right side	Week 3	18-22
155	100	Edge of test site	Weeks 1-3	19-21
123	500	Edge of test site	Week 1	---
130	500	Right foreleg	Week 3	---
142	500	Left side	Week 3	19-22
109	2,000	Right side	Weeks 1-3	9-20
170	2,000	Test site	Weeks 1-3	10-20

Body Weights:

Mean body weight for male rabbits receiving 2000 mg/kg was statistically significantly ($p < 0.05$) less than for control males for the 4, 5 and 6 semi-weekly weighings. Mean body weights for females at the same dose level were numerically less than controls but not statistically significantly lower at the 2, 3, 4, 5 and 6th semi-weekly weighing.

Body Weight Gain:

Statistically significant decreases ($p < 0.05$) were observed for males receiving 2000 mg/kg at all weighing intervals. Additionally, the data appears to indicate a log-dose response decrease in body weight gain for each succeeding time interval. Weight gains for females receiving 2000 mg/kg were statistically significantly lower ($p < 0.05$) at weighings 2 and 4. A log-dose response decrease appeared to be evident with increased dose at all time intervals.

Food Consumption:

Evidence for decreased food consumption for males was apparent at dose levels of 500 and 2000 mg/kg with the severity of the decrease very pronounced at 2000 mg/kg. Substantially decreased values were noted for females at the high (2000 mg/kg) dose group for all intervals.

Hematology and Clinical Chemistry - Both Sexes:

No log-dose responses were observed. Statistically significant decreases were randomly occurring where reported. Compound related effects were not readily apparent.

Organ Weight:

Significant differences ($p < 0.05$) between treatment and control males were observed. Male absolute heart weights for animals receiving 100, 500 and 2000 mg/kg were decreased compared with controls. Additionally, the heart/brain weight ratio decreased compared to controls with increased dose and was significantly less than controls for the 500 and 2000 mg/kg grup.

Group 4 (2000 mg/kg) mean thyroid and parathyroid/brain weight ratio was significantly decreased compared with controls. Brain/body weight and kidney/body weight ratios increased with increased dose.

No significant differences or trends between treatment groups and control for females were observed for absolute organ weights or organ weight ratios.

Histopathology:

Morphologic lesions observed at the treatment site of both treated and untreated rabbits included acanthosis, hyperkeratosis, acute and chronic dermal inflammation and epidermal ulceration. The above cited lesions were observed at a higher frequency of occurrence and at a slightly higher severity rate in the rabbits exposed to the test material. A lesion observed only in rabbits exposed to the test material (ZR-3210 Technical Half-Resolved) was dermal fibrosis. Dermal lesions observed in locations other than at the treatment site were observed in one animal of each treatment group.

Other lesions observed were considered incidental. These lesions appeared un-related by type, incidence, and severity to dermal application of the test material.

We also point out here that no adverse histopathological data was found for the heart, brain, kidney, thyroid and parathyroids that could be attributed to compound administration.

Discussion and Conclusion:

ZR-3210 Technical Half-Resolved produced a decreased consumption of food and a concurrent decrease in body weight and weight gain at 500 and 2000 mg/kg. The cause of the decreased food consumption was attributed to local irritation produced by the test chemical and the shampooing procedure inducing discomfort and a decreased desire to eat. This rationale appears to be plausible based upon the lack of adverse effects reported for clinical chemistry, hematology and histopathology (other than skin changes at the test site).

We also point out here that the changes observed for organ weights and organ weight ratios were not confirmed by the hematology, clinical chemistry or microscopic examination of tissue. The increase in heart weight may therfore be attributed to technical inconsistency in extirpating the organ. Additionally, it is also pointed out here that females showed no significant differences or trends between treatment groups and controls for absolute organ weight changes and organ weight ratios.

This reviewer has also concluded that a minimal erythema and edema appeared to be present at the lowest dose of 100 mg/kg and that up to and including 2000 mg/kg a Draize Score of 1-2 for both edema and erythema (i.e. very slight to well defined erythema and a barely perceptible to slight edema) appeared to be generally evident.

The author's of the submitted report concluded that the NOEL for ZR-3210 Technical (Half-Resolved) based upon dermal irritation, body weights, feed consumption and organ weights was 100 mg/kg.

This reviewer generally agrees with the submitted conclusions of the report. However, this reviewer would establish the dose level of 100 mg/kg as a minimal effect level for dermal irritation.

We agree however with the conclusion of the report that in context of the parameters measured a systemic NOEL of 100 mg/kg appears justified.

Classification: Core Guideline

NOTE: It is written in the report that skin sores caused from rabbits biting themselves were observed in eight (8) animals. Sores were located adjacent to the test site on three animals, at the test site on one animal and at a non-treated site on four animals.

We would also direct the reader to the results of the 90-day and 180-day dog study (oral gavage; compound administered by capsule). Dogs were observed scrathing and biting themselves after compound administration. Animals were also in almost a continuous state of vomiting. One theory presented for the biting and scratching seen in the dog study was that the vomitus contained some of the active ingredient which may have contaminated the dogs which then resulted in an irritation followed by a biting and scratching of the contaminated site.

Although we draw no conclusion between the 2 studies as to this observation we do take the opportunity to point out this apparent similarity.

#ml5-D4

00225

Subject: Pilot Rabbit Teratology Study. Final Report. Final Review.

Test Compound: ZR-3210 Technical (Half-Resolved)
Mavrik(R) Technical
Fluvalinate

Accession No: 070663

Testing Facility: Hazleton Laboratories America, Inc.

Project No.: 777-136

Responsible Professionals:

Gary W. Wolfe - Study Director and Laboratory Supervisior

Ruth S. Durloo - Research Associate, Toxicology Department

Robin B. Phipps - Technical Writer

Testing Period: April 15, 1981 - May 14, 1981 (day of cesarean sections)

Report Submitted to Sponsor: August 21, 1981

Purity of Test Material: 93.1%

Batch or Lot No.: Run 23-R Analysis No. 0281037.

Homogeneity, Stability, Concentration:

The stability data through six months for fluvalinate technical (half-resolved, Run 23-R) at temperatures of 42° and 50°C would allow the projection of 90% or better of the initial value for two years at 25°C. The material appears to be very stable when kept in sealed glass jars.

Fluvalinate (half-resolved) dosing solutions in a corn oil vehicle prepared at and received from Hazleton Laboratories were also analyzed using GC and HPLC. The results of duplicate preparations of four sample were similar. The mean values of the samples analyzed were within acceptable limits of the target concentration.

Materials and Methods: Seventy-five females and fifty-four male New Zealand White rabbits (males are maintained as breeding stock and serve as sperm donors for the artificial insemination of the does) were received from Dutchland Animals, Inc., Denver, Pennsylvania on February 16, 1981. All animals were individually housed in stainless steel cages, uniquely identified by metal ear tags and acclimated to laboratory conditions for two months prior to initiation of the study. Purina Lab Rabbit Chow(R) and tap water (via water bottles) were available ad libitum. Animal quarters were controlled for temperature, humidity, and a 12-hour light/dark cycle.

Following the acclimation period and health status examination by a staff veterinarian 20 females were randomly assigned (HLA computerized randomization

process) into 5 groups of 4 females per group. Group 1 (controls) received only Dukes(R) Pure Corn Oil (C.F. Sauer Company, Richmond, Virginia). Groups 2 through 5 received the test compound in the corn oil vehicle at levels of 10, 50, 250, and 1000 mg/kg/day once a day by oral intubation from day 6 through day 18 of gestation. All dosages were adjusted to 100 percent of active ingredient and administered on a weight per weight basis. Dosing factors were 2.0 ml/kg for groups 1, 3, and 5; 0.4 ml/kg for Group 2; and 0.5 ml for group 4. The dosing factors were based on individual body weights on day 6 of gestation.

Ovulation was induced in the test animals by I.V. administration of 250 I.U. of human chorionic gonadotropin (HCG) hormone (A.P.L.(R) Control No. 1 ZCP, manufactured by Ayerst Laboratories, Inc., New York, New York) into the marginal ear vein. Approximately six (6) hours following injection of HCG, the does were artificially inseminated using a modification of the method of Gibson (1966). The does were inseminated a second time, approximately one and a half-hours later. The day of insemination was designated as day 0 of gestation.

All does were observed twice daily for mortality and moribundity and once daily for pharmacological and toxicological effects. All does were weighed on days 0, 6, 11, 15, 18, 24, and 29 of gestation. A gross inspection of food consumption was made daily.

All surviving females were sacrificed by T-61(R) euthanasia solution (Taylor Pharmacal Company, Decatur, Illinois) and the fetuses taken by cesarean section on Day 29 of gestation. Following gross examination of each dam, the uterus and ovaries were excised, weighed (uterine weight of several does inadvertently not recorded), and examined for the number of corpora lutea per ovary and the number and placement of uterine implantation sites, early and late resorptions, and live and dead fetuses in each uterine horn. Fetuses were removed from the placenta, individually identified, examined externally, and weighed and measured from the frontal-parietal suture to the base of the tail (crown-rump distance). Cesarean sections were also performed on animals that were found dead or sacrificed prior to day 29 of gestation and the number of corpora lutea, implanations, and resorptions were recorded if observed.

The uterus and ovaries of each dam were preserved in 10% neutral buffered formalin. The fetuses were frozen for possible future reference.

Results - Maternal Data. Six animals were found dead or sacrificed prior to termination of the study -- one from control group, two from group four, and three from group five. Two of these deaths occurred after dosing and were probably a result of technical errors (i.e., accidental intratracheal injection). The control animal died on day 6 and an oil-like fluid was contained in the thoracic cavity. One group four animal died on day 16 and the lungs contained a yellow purulent material. In addition, two of these animals (one in group four and one in group five) were sacrificed following signs of an abortion (placenta-like substance found in the trays).

Anorexia was observed in most control and treated animals during and after the dosing period. The predominant sign noted in treated animals was depression which occurred during the dosing period and was also observed during the

post-treatment period. Soft stools, labored respiration, and ataxia were observed in treated animals during the dosing period. During the post-treatment period these signs disappeared. Animals showing signs during the post-treatment period were not the same animals showing signs during the period of dosing.

Weight loss was noted in all groups (except group 2) during the dosing period (days 6-18). Body weight loss was most severe in the high-dose group (twice that of controls) during days 6-18 and substantially lower than controls for days 18-29.

Gross necropsy findings were primarily observed in animals found dead or sacrificed prior to day 29 of gestation. It was reported that findings were generally unremarkable (except as noted above) and not unlike those commonly observed in New Zealand White rabbits necropsied in the contractor's laboratory.

Results - Cesarean Data: Pregnancy rates were comparable between the control and all treated groups (75% for controls and 75-100% for treated groups). The survival rate for pregnant does was 100% for groups 1, 2, and 3 but only 33% for groups 4 and 5.

The mean number of corpora lutea was comparable among the treated groups (9.8 - 11.3) and considerably lower in the control group (5.3). However, the mean implantation efficiency was higher in the control group (88%) than the treated groups (69-79%). The number of resorptions were comparable between the control, the low-, and the low mid-dose group. No dead fetuses were observed in these groups. The mean number of live fetuses were comparable between the low-dose (10 mg/kg) group and the low mid-dose group (50 mg/kg) (a mean of 6.3 vs. a mean of 5.3 live fetuses). The mean number of live-born fetuses in the control group was 1.7. Mean values were not recorded for the high mid-dose and high-dose groups, as only one litter of nine pups was delivered in the high mid-dose group and 100% of the implants were resorbed in the high dose group. Mean body weights and mean crown-rump distance values were comparable between the control group and groups 2, 3, and 4. No fetuses were available for evaluation in the high dose group due to the total resorption of implants.

Visceral evaluation of fetuses indicated that all fetuses appeared normal with the exception of one fetus (50 mg/kg) which had pale kidneys and dilation of the renal pelvis.

Conclusion: Oral administration of half-resolved ZR-3210 technical (fluvalinate) at levels of 10, 50, 250, and 1000 mg/kg to pregnant rabbits from days 6-18 of gestation produced maternal toxicity and embryotoxicity at levels of 250 and 1000 mg/kg, while the levels of 10 and 50 mg/kg were without apparent effect. On the basis of the data, the dosage levels of 0, 5, 25, and 125 mg/kg were chosen for the expanded teratology study.

Classification: Supplementary.

Subject: Rabbit Teratology Study. Final Report. Final Review.

Test Compound: ZR-3210 Technical (Half-Resolved)
Mavrik(R) Technical
Fluvalinate

Accession No: 070663

Testing Facility: Hazleton Laboratories America, Inc.

Project No.: 777-137

Responsible Professionals:

Ruth S. Durloo - Research Associate, Toxicology Department

D. Keith Pruett - Technical Writer

Gary W. Wolfe - Project Coordinator, Toxicology Department

Frederick G. Snyder - Manager, Office of Quality Assurance

Testing Period: May 26, 1981 - June 26, 1981

Report Submitted to Sponsor: December 23, 1981

Purity of Test Material: 93.1%

Batch or Lot No.: Run 23-R, Analysis No. 0281037.

Homogeneity, Stability, Concentration:

The stability data through six months for fluvalinate technical (half-resolved, Run 23-R) at temperatures of 42° and 50°C would allow the projection of 90% or better of the initial value for two years at 25°C. The material appears to be very stable when kept in sealed glass jars.

Fluvalinate (half-resolved) dosing solutions in a corn oil vehicle prepared at and received from Hazleton Laboratories were also analyzed using GC and HPLC. The results of duplicate preparations of four samples were similar. The mean values of the samples analyzed were within acceptable limits of the target concentration.

Materials and Methods: Ninety female New Zealand White rabbits were received from Dutchland Laboratory Animals, Inc., Denver, Pennsylvania on March 30, 1981. All animals were individually housed in stainless steel cages, uniquely identified by metal ear tags and acclimated to laboratory conditions for two months prior to initiation of the study. Purina Lab Rabbit Chow(R) and tap water were available ad libitum. Animal quarters were controlled for temperature, humidity, and a 12-hour light/dark cycle.

Following the acclimation period and health status examination by a staff veterinarian, 68 females (weight range 3390 to 5200 grams) were randomly as-

signed into four groups of 17 does per group. Group 1 (controls) received only Dukes(R) Pure Corn Oil (C.F. Sauer Company, Richmond, Virginia). Groups 2 through 4 received the test compound in the corn oil vehicle at levels of 5, 25, and 125 mg/kg/day once a day by oral intubation from day 6 through day 18 of gestation. All dosages were adjusted to 100 percent of active ingredient (as indicated in the pilot study) and administered on a weight per weight basis. All test animals were dosed once daily on days 6 thru 18 of gestation at a constant volume of 1.0 ml/kg. of body weight. The dosing volumes for each animal for days 6 through 18 were based on the individual body weights recorded on day 6 of gestation.

[NOTE: On day 15 only, half of the mid-dose and half of the high-dose female dosages were based on individual day 15 body weight values. The misdosed mid-dosed females were dosed at a mean of 25.20 mg/kg and the misdosed high-dose females were dosed at a mean of 108.72 mg/kg. Subsequent dosage calculations day 16 through day 18 of gestation were based on day 6 body weights.]

Ovulation in the test animals was induced by I.V. administration of 0.5 ml (250 I.U.) human chorionic gonadotropin (HCG)(A.P.L(R) Control No. 1 ZCP, manufactured by Ayerst Laboratories, Inc., New York, New York) into the ear vein. Approximately six hours following the injection of HCG, the does were artificially inseminated using a modification of the method of Gibson (1966). The does were inseminated a second time approximately one hour later. The day of insemination was designated as day 0 of gestation. Half the animals were inseminated on May 26, 1981, the remainder on May 28, 1981.

All animals were observed twice daily for mortality and moribundity from receipt through study termination. Daily observations for pharmacological and toxicological effects were made during gestation days 0-29. All females were weighed on days 0, 6, 11, 15, 18, 24, and 29 of gestation. A gross inspection of food and water consumption was made daily.

On day 29 of gestation, all surviving females were weighed, sacrificed by T-61(R) euthanasia solution, and the fetuses were delivered by cesarean section. Following gross examination of each doe, the number of corpora lutea per ovary, uterine weights (with and without fetuses), and the number and placement of implantation sites, early and late resorptions, and live and dead fetuses in each uterine horn were recorded. Fetuses were removed from the placenta, individually identified, examined externally, weighed, and measured from the frontal-parietal suture to the base of the tail (crown-rump distance). Cesarean sections were also performed on animals that were found dead or delivered prematurely and the number of corpora lutea, implantations, resorptions, and fetuses were recorded if observed.

Fetuses were examined externally and then opened by longitudinal incision, sexed, and the viscera were examined in the fresh state using Staple's technique (Staples and Schnell, 1964). The heads from approximately one-third of the fetuses from each litter were removed and fixed in Bouin's solution, sectioned, and examined by Wilson's freehand razor technique (Wilson and Warkany, 1965) and sealed in plastic. The prepared sections of eyes, palate, nasal septum, and brain were reexamined with the aid of magnification.

Following visceral examination, all fetuses were eviscerated, skinned, and processed for skeletal evaluation. After proper fixation and dehydration, the skeletons were stained in a potassium hydroxide-alizarin red S solution, cleared in Mall's solution and glycerol-ethanol-benzyl alcohol (2:2:1), and stored in a glycerine-ethanol solution (1:1). Each skeleton was examined on a light box with the aid of magnification for bone alignment, degree of ossification, and anomalies. The number of sternebrae, ribs, caudal vertebrae and bones of the extremities was noted and recorded.

The mean maternal body weight changes (days 0-6, 6-18, 18-29, and 0-29) and mean fetal body weights (by sex) and lengths (by sex) of the control group were compared statistically to the treated groups by Bartlett's test for homogeneity of variance and the one-way classification analysis of variance. If the analysis of variance of homogeneous data was significant, Scheffe's multiple pairwise comparison procedure was used to compare the group mean values.

Pregnancy rates were analyzed by Fischer's Exact Test for proportions (one-tailed).

Percent males per litter, implantation efficiency, incidence of resorptions, fetal viability, and incidence of fetal skeletal and visceral anomalies and variants were analyzed by the Kruskal-Wallis nonparametric analysis.

All analyses were evaluated at the 5% probability (one-tailed) level.

Values statistically higher and lower than control values were indicated as S+ and S-, respectively.

Results - Maternal Data: Survival was comparable between the control and treated groups. One pregnant control animal died during dosing on day 10 of gestation and one pregnant high-dose female was found dead on day 16 of gestation. Death of the control animal was attributable to an error in dosing technique as suggested by the gross necropsy findings. The cause of death in the high-dose animal was not readily apparent. One control animal and one high-dose female were both sacrificed near the end of "term" after discovery of signs indicating abortion. No unusual gross pathology was observed in either animal. Anorexia, depression, and discharge from the eye were the predominant signs observed. Anorexia in pregnant does was observed in controls and the lowand mid-dosed groups to an equivalent extent. Anorexia in the high-dose group was observed 2 to 2.5 times more than any other group. Depression was also observed between treated and control groups but occurred 5 to 10 times more often in the high-dose group when compared to the lower dosed groups and controls, respectively. Some correlation appeared to be evident between the anorexia observed and the depression which was manifested at the highest dose level. Two signs appearing at the highest dose level but not in any other group were matted hair coat and labored respiration (1 observation each). Discharge from the eye was also observed with increased dose. Discharge from the eye (epiphora, one or both eyes) was observed once in the control group and 8, 21 (same animal), and 15 times in the low to the progressively higher doses, respectively. However, ocular discharge was noted prior to the initiation of the study in some animals (various groups) with no further occurrence noted during the study for the same animals. Other animals

(all groups) exhibited signs of ocular discharge prior to the initiation of the study and these same animals showed discharge during the study. The ocular discharge therefore does not appear to be compound-related.

Mean maternal body weight changes were generally comparable between all groups on gestation day 0 through 6. However, during the period of dosing (days 6-18), the high-dose group manifested a statistically significant body weight decrease which was approximately 10 times greater than in any of the other groups. Body weight decreases for controls and the lowand mid-dose groups were generally similar and ranged between 62 and 84 grams. The cessation of dosing resulted in body weight gains, with the greatest gain occurring in the high-dose group. The gain was statistically significant, being 4 times greater than the lower dose levels and approximately 20 times greater than controls. For the entire gestational period (days 0-29), only the high-dose group manifested a substantial and statistically significant mean body weight decrease.

Gross pathology of does found dead or sacrificed was not remarkable with the exception of observations associated with the death of the animal attributed to technical error.

Results - Cesarean Data: Pregnancy rates were comparable between controls and the midand high-dosed animals. The pregnancy rate for the low-dose group was statistically significantly lower than controls. However, as this decrease was not log-dose-responsive at the higher doses, the result was considered not to be compound-related.

The mean values for corpora lutea, implantations, resorptions, and dead/live fetuses were not statistically significant between treated groups and controls. However, a slight downward trend with an increased dose was noted with respect to the number of implanations and the number of fetuses born alive. A slight increase in the number of resorptions was also observed with an increased dose. The mean implantation efficiency and the mean incidence of resorption (percentage) were also calculated and reported on a per litter basis. No statistically significant differences were recorded between groups for either parameter. However, the mean incidence of resorption was greater by two-fold in the high dose group than in any other group (20% vs. 40%).

No statistically significant differences were recorded between groups for fetal mortality or for fetal viability. However, viability for the high-dose group was 16% less than in any of the other groups all of which were comparable on a percentage basis.

The mean body weight (grams) and mean crown-rump distance (centimeters) for live males and females were generally comparable to control values.

The mean percentage of males born per litter ranged between 42 and 55 percent.

Gross pathological findings in the control, low-, and mid dose groups were not remarkable. Three fetuses manifested protruding intestines, and four had front paws rotated inward in the high-dose group. The protruding intestines can be considered as not compound related as these findings have been observed

in control fetuses. Four fetuses from a single litter of the high-dose group had front paws rotated inward. It was reported that this finding was observed in control animals of previous studies. It was the contractor's opinion that the paws would have grown in a normal manner if the animals had been allowed to mature.

The number and incidence of visceral anomalies and variants were not statistically different between groups. However, there was an increase in the incidence of visceral anomalies observed in the high-dose group. The anomalies were limited to 2 litters and included gastroschisis (i.e., rupture or fissure of the abdominal wall with some intestinal protusion), short intestinal tract, a septal defect, and one eye small and filled with bloody fluid. It was reported that findings similar to these have been observed in control rabbit fetuses.

The incidence of skeletal anomalies were increased in the high-dose group as a result of fetuses in one litter having short and spatulate ribs (5 rabbits) short and curved femurs, (5 rabbits), and curved tibia and fibula (4 rabbits). The report indicated that the anomaly not observed routinely was the curvature of the tibia and fibula. A total of 10 litters and 55 fetuses were examined at the high dose.

The incidence of skeletal variants was reported to be highest in the control group, with treated groups generally comparable to each other.

Discussion: Anorexia, general depression, and decreased body weight gain (clearly expressed during the period of dosing) appear to be interrelated events and are considered by this reviewer to be definitive expressions of maternal toxicity in the high-dose group. Accompanying the maternal toxicity in the high dose group was an embryo or fetotoxic effect, as indicated by a lower implantation efficiency, higher incidence of [illegible]rption, and concurrent lower fetal viability when values were compared to the control group. Although not statistically significant from controls, the differences in values were large and consistent, and are considered to be related to the administration of compound and a secondary effect of maternal toxicity. The visceral anomalies reported for the high-dose group (gastroschisis, short but complete intestinal tract, septal defect (heart), one eye small and filled with bloody fluid) have reportedly been previously observed in control animals and were considered to be incidental. This reviewer is inclined to agree. Some focus was given to the skeletal anamolies of the curved tibia and fibula, events reported as not being observed routinely and the significance of which was not known. It was mentioned, however, by the contractor that the short spatulate ribs and the short, curved femurs occurred often enough to classify the event as fortuitous. This reviewer believes that reasonable arguments can be made that the presence of the curved tibia and fibula are likely to be non-teratogenic events. The routine presence of short and spatulate ribs, short femurs, and particularly the curved femur would seem to some extent to deemphasize the significance of the curved tibia and fibula. The fact that maternal toxicity (anorexia and depression) was also observed at this dose level may have led to the expression of a less sensitive event to which the animal was already predisposed. It would therefore seem that the likelihood of the curved tibia and fibula being a teratogenic response to the compound is less likely to be accepted as a teratogenic event. This reviewer therefore takes

the position that the event (curved tibia and fibula) is not a teratogenic expression of the compund but rather an extension of the expressions already observed.

Classification: This study is being upgraded from core-minimum (provisional) to core-guideline classification without qualification.

Summary:

125.0 mg/kg	No apparent teratological effects at 125.0 mg/kg (HDT). Maternal toxicity was in evidence. Fetotoxic effects were observed and considered to be secondary to maternal toxicity.
25.0 mg/kg	No readily apparent maternal or fetal effects at 25.0 mg/kg (mid-dose-tested).
5.0 mg/kg	No readily apparent maternal or fetal toxic effects at 5.0 mg/kg (low-dose-tested).

NOEL for terata: 125.0 mg/kg
NOEL for fetotoxicity: 25.0 mg/kg
NOEL: 5.0 mg/kg

HED/TOX:AKOCIALSKI/SBAILEY:DCR-07845:WANG-0926B:efs:Raven:479-2013:05/3/82
REVISED:05/07/82:DCR-07848:FILE-0926B
REVISED:DCR#07901:CHP:05/14/82

Subject: Two Generation Rat Reproduction Study. Final Report.

Test Compound:

ZR-3210 Technical Racemic Mixture
Fluvalimate Technical Racemic Mixture
MAVRIK Technical Racemic Mixture

Accession No: 070660

Testing Facility: International Research and Development Corp.

Study No.: 322-039

Responsible Professionals:

Barry Benson - Director of Quality Assurance
Sheryl Slezak - Report Writer
James L. Schardein - Director, Reproduction and Teratology
Malcolm Blair - Director of Toxicology Division
Patricia Lang - Study Director
Joanne Kopplin - Senior Staff Pathologist
Ward Richter - Director Pathology Division

Testing Period: January 29, 1980 - May 1, 1981

Report Submitted to Sponsor: September 29, 1981

Purity of Test Material: 93.8%

Batch or Lot Number: Run 7, Anal #0979-069

Homogeneity, Stability, and Concentration in Feed: Actual and referenced data indicated that the compound was stable and homogeneously distributed in the feed. The target test article concentrations were also within acceptable ranges.

Materials and Methods:

One hundred ninety-eight male and 198 female weanling rats were obtained from the Charles River Breeding Laboratories, Portage, Michigan. All animals were carefully observed for abnormalities during the 14 day acclimation period. Animals observed to be in poor health were sacrificed and discarded. All F-0 and F-1 parental animals were individually housed in hanging wire-mesh cages except during the mating, gestation and lactation periods. The rats were housed in a room controlled for temperature, humidity and light (12 hour light/dark cycle). Purina Certified Rodent Chow(R) #5002 and tap water were available ad libitum. The drinking water was analyzed on a quarterly basis for the presence of pesticides, heavy metals and coliform bacteria. The results are available upon request. A weight range of the mean (plus or minus 1.5 standard deviations) was used as the limit for selecting animals to be placed on the study. One hundred fifty male and 150 female rats were assigned

to one control and five treatment groups using a computer generated list of random numbers. Permanent animal numbers were assigned at this time and all animals identified by metal ear tags bearing their permanent numbers. These animals were the F-0 generation. Twenty-two (22) animals which were rejected by the randomization process were selected for a viral screening study (IRDC Study No. 322-041). The remaining animals were sacrified and discarded. The technical ZR-3210 racemic mixture was administered in the diet at dosage levels of zero (0, vehicle control, Mazola(R) Corn Oil) 20, 100, 250, 500 and 1000 ppm to the F-0 generation. Since the test diet was 93.8% pure, an adjustment factor of 1.07 was used. Diets were prepared fresh every two weeks. The F-1 and F-2 generations were potentially exposed in utero and then in diet during lactation and from weaning until sacrifice. The F-0 parental rats, after a 15 week period of treatment, were housed in units of one male to one female in hanging wire-mesh cages, and allowed 2 weeks to mate. A record of mating of the females in each of the groups was obtained by daily inspection for a copulatory plug. Males were then returned to their original cages and females were housed in plastic cages containing ground corn-cob bedding throughout gestation, delivery and lactation. Toward the end of the 21 day gestation period, females were examined twice daily for signs of parturition. The day that the last pup in the litter was delivered was defined as day 0 (zero) of lactation. The litters were examined as soon as possible after delivery for litter size, stillborns, live births, sex and any gross anomalies. Litters were reduced to 10 pups of equal sex, if possible, on lactation day 4. Pups were housed in with their dams for 3 weeks after birth and remained with their litter mates for a minimum of 3 days after weaning. Twenty-five males and 25 females from each group were then selected using a table of random numbers with no more than two (2) pups of each sex per litter to become parents of the next generation (F-1 parents). Records of the selection procedure were maintained to avoid sibling matings in the F-1 generation. Three weeks after the mating period, 10 randomly selected males from each group that had successfully inseminated females were sacrified and necropsied. The remaining males were sacrificed at this time, but not necropsied, with the exception that all F-0 (as well as F-1 adult males) males were examined for spermatogenesis upon sacrifice. Upon sacrifice, the testes and epididymides were removed, trimmed and the testes were weighed. The semen was then exmained microscopically at 400X in a solution of 0.9% physiological saline. Since no apparent gross abnormalities were observed during the F-0 spermatogenesis evaluation, histopathological examination of the testes was not conducted. The testes of all F-1 males were saved in 10% formalin for future microscopic examination. All F-0 females were externally examined and necropsied, after selection of F-1 pups to become parents of the next generation. The uteri of all females which did not deliver were opened and placed in 10% ammonium sulfide solution and examined for pregnancy status. Any condition which could have prevented pregnancy was recorded.

Pups from the F-1 and F-2 generation (F2a, F2b litters), 5 per sex per dose, were randomly chosen for necroposy. The F-1 pups were chosen after selection of F-1 parental animals.

All pups not selected for necropsy or to become parental animals were sacrificed and discarded. All animals (scheduled, and unscheduled sacrifices as well as those found dead) were given a complete post-mortem examination under the direct supervision of a pathologist.

After a thorough external examination, each animal was opened and the contents of the abdominal, thoracic and cranial cavities were examined both in situ and after dissection. Any macroscopic abnormalities observed were recorded on the Pathology Record.

The following tissues from F_0, F_1 parental, F_1 pup and F_{2a} and F_{2b} generation animals were trimmed free of fat and connective tissues and weighed at time of post-mortem examination with weights recorded prior to placement in fixative:

adrenal (2)*	brain
heart	spleen
kidney (2)	testes
liver	thyroid/parathyroid complex*

*after fixation

The following tissues from the F_0, F_1 parental, F_1 pups and F_{2a} and F_{2b} generations were collected and placed in phosphate-buffered neutral formalin for fixation:

adrenal (2)	pituitary
aorta	sciatic nerve
brain	prostate
colon	salivary gland
duodenum	seminal vesicles
esophagus	skeletal muscle including sarcolemma
eyes	skin (including mammary gland)
heart	spleen
ileum	spinal cord (3 levels)
jejunum	stomach
kidney (both)	testes - with epididymis
liver	thymus
lungs	thyroid
lymph node (mesenteric)	trachea
mammary gland	urinary bladder
ovaries	uterus
pancreas	cervix
	all macroscopic lesions

Histopathology

Hematoxylin and eosin stained paraffin sections of the following tissues were prepared and examined microscopically by an IRDC staff pathologist for the F_1 adults (10/sex/group), F_1 weanlings (5/sex/group), and F_{2a} weanlings (5/sex/group).

adrenals (both	spinal cord (3 levels)
colon	spleen
heart	stomach
ileum	seminal vesicles
jejunum	testes - with epididymis
kidney (both)	thyroid - with a section of trachea and

liver (2 sections)	esophagus
lungs	urinary bladder
brain	prostate
peripheral nerve (sciatic)	uterus
pituitary	cervix
ovaries	skin (mammary gland)
skeletal muscle including sarcolemma	all other gross lesions

One animal, #45067AF, was not scheduled for histopathology. A gross lesion was observed in the uterus and processed for histologic evaluation.

The F-0 generation provided the information to establish 3 treatment levels to be continued in the F-1 generation. The 1000 ppm group was terminated because of excessive toxicity, whereas the 250 ppm group was considered a "minimal-effect" level. Therefore, levels of 0, 20, 100, and 500 ppm were selected for administration to the F-1 generation.

The F-1 parental animals were selected from the F-1 litters at approximately 24 days of age. All F-1 pups were available for selection except those not expected to survive because of physical abnormalities. Brother-sister matings were avoided. Animal care and treatment was as previously described. After a minimum of 120 days animals of equivalent dose levels were mated. For the first F-1 mating, evidence of copulation was determined by detection of a copulatory plug. A maximum of 10 days was allowed for mating with the first male. If no evidence of copulation was observed within 10 days the female was mated with a proven male (defined as a male that had successfully inseminated one female) from the same treatment group for an additional 5 days. Since the number of confirmed matings was low after the initial 15 days, the mating period was extended for an additional 5 days using a second proven male from the same treatment group. Due to a reduced number of litters in all groups, including the control, from the first mating and to ensure an adequate number of litters, the protocol was amended to add a second mating of F-1 parental animals (thus producing an F-2b litter). In an effort to find the cause of the reduced fertility during the first F-1 mating, females were paired for the first 10 days of the second F-1 matings with the same male used for the first mating. The males used for remating were from the same treatment group as the female and were proven fertile during the first F-1 mating. Positive vaginal smears for sperm were relied upon to document insemination of F-1 females for the second F-1 mating (producing the F-2b generation). When insemination was established, the procedures followed during gestation and lactation were the same for the first and second F-1 mating and the resultant litters. All females which did not deliver F2b litters were examined by daily vaginal smears to evaluate estrous cycles for two weeks in an effort to determine why many F-1 females did not deliver following evidence of mating.

The entire study was conducted for 66 weeks; the F-0 generation from study weeks 1 thru 24 and the F-1 generation from week 25 thru 66.

General Observations:

The parental rats and their pups were observed twice each day for signs of overt toxicity, changes in general appearance and behavior, and mortality. Detailed observations were recorded weekly on the parental rats. In addition, detailed observations were performed on the pups after weaning. Examinations for gross abnormalities of pups were conducted at birth, and on lactation days 4, 7, 14 and 21. Individual body weights were recorded weekly for all adult animals. Female body weights during gestation and lactation were recorded on days 0, 6, 15 and 20 of gestation, and days 0, 7, 14 and 21 of lactation. Pups were weighed individually on days 0, 4 (before and after reduction), 7, 14 and 21 of lactation. Parental food consumption was measured weekly for individual rats of the F-0 generation from study initation until mating. Food consumption of the F-1 generation was measured weekly between the time of weaning and mating. Individual food consumption could not be measured during the mating periods due to cohabitation. However, the animals remained on the test diet at the appropriate concentrations during this time. Individual food consumption for maternal animals was measured on days 0, 6, 15 and 20 of gestation and days 0, 7, 1- and 21 of lactation. The reproduction parameters observed for this study wei .le and female fertility, gestation length, and parturition. These parameters were assessed for all matings. Litter evaluations included pup viability at birth, pup survival during lactation, newborn body weight, and pup growth during lactation. All statistical analysis compared the treatment groups with the control group for significant differences at the $p < 0.05$ and $p < 0.01$ levels. Parental body weights by sex were analyzed by one-way analysis of variance, Bartlett's test for homogeneity of variances, and the appropriate "t-test" (for equal and unequal variances) as described by Steel and Torrie. Significant differences were determined using Dunnett's multiple comparison tables. Analyses were conducted at one week prior to mating (week 15) and the last week of the generation (week 20 for males, week 24 for females) for the F-0 generation. Body weights for the F-1 parental animals were analyzed at the first week of the generation (week 25) the week before mating (week 40) and the last week of the generation (week 66). Male and female fertility indices were compared using the Chi-square test criterion with Yate's correction for 2 X 2 contingency tables and/or Fisher's exact probability test as described by Siegel to judge levels of significant differences. The proportion of live pups at birth per total number born and the survival indices at lactation days 0, 4, 7, 14 and 21 were compared by the Mann-Whitney U-test as described by Siegel and Weil to judge significant differences. The mean numbers of live born pups per litter and mean body weights of pups and absolute and relative (to both body and brain) organ weights (F-0, F-1, F-2a, F-2b) were analyzed by one-way analysis of variance, Bartlett's test for homogeneity variances and the appropriate "t-test" (for equal or unequal variances) as described by Steel and Torrie. Significant differences were determined using Dunnett's multiple comparison tables. Mean pup body weights on lactation day 21 were analyzed by sex.

Results: General Behavior, Appearance and Mortality.

F-0 Parents

It was reported that hair loss was increased in those groups of females receiving 250, 500, and 1000 ppm of compound during study weeks 9 thru 24. In males, increased hair loss was confined to the 500 and 1000 ppm dosage groups during study weeks 9 thru 20. The presence of matted hair coats was observed in females, primarily at the high dose level. Matted hair coats were not observed at any time in males. Open sores were observed in males and females in the 100 thru and including 1000 ppm dosage groups, with the greatest incidence (13 males and 7 females) observed in the 1000 ppm dosage group. Observations of open sores began on study week 12 and continued throughout the generation. Scabbing was observed on all animals with open sores and was generally found on the head, neck, shoulders, forelimbs and ears of the animals. It was reported that the frequency of scabbing increased with increasing dosage in the male animals, while in females it increased with increased dosage only in the 100 to 500 ppm dosage groups. Thirty-six (36) observations of scabbing were noted in the 500 ppm dosage group females, as compared to 33 in the 1000 ppm dosage group females.

Swollen, dry or cloudy eyes were observed in both males and females during the first week of the study. The observation was not observed at all dosage levels, and during the following study weeks the observations for this condition, in the animals of both sexes, were reported as not biologically meaningful at any dosage level.

Only two inter-current deaths were reported, one female in the control group and one male in the 1000 ppm group. One female in the 250 ppm dosage group and one female in the 500 ppm dosage each had a subcutaneous mass in the lateral neck area. These masses were not examined histologically.

The ratio of females having no evidence of insemination and not delivering a litter to those showing evidence of insemination and not delivering a litter appeared to be constant between all groups.

Necropsies performed on females that did not deliver revealed that one dam was gravid with one early resorption. No abnormalities were observed in the nongravid females.

Necropsy of F-0 males at the terminal sacrifice revealed that at 250 ppm and above there was hair loss, necrotic skin, scabbing, and open sores. The observations did not appear to be log-dose responsive, although the observed incidences were greater in treated animals than in controls. Mandibular and axillary lymph modes were enlarged at 250 and 1000 ppm. Macroscopic observations of F-0 females showed some hair loss at all dose levels including controls, with a greater incidence noted at 250 ppm and above. Submandibular, axillary and mandibular lymph modes were enlarged at 250 ppm and above. Scabbed and necrotic areas were observed at 500 ppm and 1000 ppm.

A slight decrease in food consumption was observed in the 250, 500 and 1000 ppm dosage groups for males at 20 weeks. Females at 15 weeks showed comparable food consumption values for all treated groups although values were slightly lower than controls.

Decreased mean male body weights were statistically significant during weeks 15 and 20 in the 500 and 1000 ppm dosage groups. Statistically significant decreases in female body weights (1000 ppm) occurred during weeks 15 and 24.

During gestation days 15-20, apparent dose-related decreases in mean maternal body weight change were observed in the 500 and 1000 ppm dose groups (-18 and -37% respectively). During lactation maternal body weight changes appeared, at times, to be erratic. However, treated maternal groups appeared to gain more weight than the control group. This was generally reflected as positive weight gains for treated mothers over controls during the 7-14 day period, and substantially less weight loss with increasing dose during the 14-21 day period. Less maternal weight loss with increasing dose for the 14-21 day period appeared to be related to dose.

Female food consumption expressed as g/kg/day was decreased during the entire lactation period in the 1000 ppm group and during days 7-21 of the 500 ppm group. The decreases appear to be treatment related.

Organ to body weight ratios revealed statistical differences for all organs weighed; however, these differences disappeared when organ to brain weight ratios were taken. The heart body weight-brain weight ratio was, however, statistically significantly lower at 500 and 1000 ppm for F-0 females. The biological interpretation of this event is not readily apparent.

F-1 Parents

No abnormal behavior of the parental animals was observed during study weeks 25 thru 66.

Skin lesions were observed in F-1 animals. In the 10 weeks after weaning, animals in the 100 ppm and 500 ppm dosage levels had increased hair loss, sores and scabbing on the head, neck and shoulders; both the frequency and severity of these observations were increased in the 500 ppm group. During the time course of the experiment the frequency, severity, presence and absence of the lesions fluctuated. However, at the time of necropsy the evidence for the presence of lesions was greatly diminished. Prior to their sacrifice and discard on study week 25, the observations for the animals in the 250 and 1000 ppm dosage groups were similar to the 100 and 500 ppm groups. Only 1 control F-1 male had skin lesions.

Cloudy eyes as observed in some F-0 animals were observed in only one F-1 male.

Swelling of the ventral neck area was observed in F-1 parental animals in all groups including controls during weeks 54-60. Swelling persisted for approximately 1-2 weeks, and was likely due to a viral infection.

In the 1000 ppm group, 15 males and 18 females of those selected to potentially become F-1 parental animals died between the time of selection and selection of dose levels for initiation of the F-1 generation. The dead animals were in the age range of 25 to 32 days. Five of the 18 dead females

had necropsy observations indicating red or black foci in the glandular mucosa of the stomach. Male necropsies were unremarkable. All animals in the 1000 and 250 ppm dose groups were eventually sacrificed and discarded from the study on toxicological considerations. This was previously noted in the earlier part of this report.

During the remaining weeks of the F-1 generation, 2 males and 1 female died in the 500 ppm dosage group. Survival was 100% in the 0, 20, and 100 ppm dosage group.

Body weights of the treated males were consistently less than body weights of the control animals during the entire F-1 generation (weeks 25-66) with body weight differences between males in the 500 ppm group 18% less than controls and statistically significant at a level of $p < 0.01$ at 66 weeks. Additionally, statistically significant decreases were also recorded for study weeks 25 and 40 for the 500 ppm dose groups. Female body weights at week 66 were very comparable between the control group and the 2 low dose groups. The high dose female group showed a 6% body weight decrease at week 66.

Mean maternal body weights were lower for the first mating of the F-1 generation to produce the F-2a generation than control values during gestation days 0-6; however, no log-dose response increase was observed. A dose level of 500 ppm showed a generally uniform maternal body weight decrease (ca. 25%) for all time periods. Maternal body weight changes during lactation, although somewhat erratic, appeared to be gener lly comparable between control and treated groups. For the second F-1 mating to produce the F-2b generation the 100 and 500 ppm dosage groups had mean maternal body weights comparable to the control group during gestation and lactation.

Food consumption values for the F-1 parent generation, for the entire period (weeks 25-66), were slightly decreased for males at 500 ppm. The values were not, however, statistically significant. Food consumption for females was similar between treated groups and controls.

Females had decreased food consumption during lactation days 0 through 21 in the first mating in the 500 ppm group. This decrease was not observed in the second F-1 mating and therefore may or may not be related to treatment. Food consumption values for parental rats were comparable to controls during gestation and lactation at doses of 500 ppm and lower.

Reproductive Parameters

Fertility: Insemination and Delivery (F-0 Mating) to Produce the F-1 Generation.

Parameter	Dose ppm					
	0	20	100	250	500	1000
Females paired with males (%)*	100	100	100	100	100	100
Total of females that delivered (%)	84	68	80	72	72	80
Females with evidence of insemination (%) (copulatory plug)	76	60	60	68	64	68
Number of females which did not deliver	1	6	4	4	5	3

* 25 Females per group

Prior to termination of the F-0 generation, the uterus of each female not delivering was examined. No significant findings were observed.

Sperm evaluation conducted on the F-0 males at sacrifice revealed either no motility or reduced motility as follows:

Dose (ppm)	0	20	100	250	500	1000
Motility (reduced or none)	1	6	7	9	8	8

However, of those animals manifesting reduced or no motility, successful insemination was observed at the following doses:

Dose (ppm)	0	20	100	250	500	1000
Successful insemination	1	6	5	6	6	6

Fertility: Insemination and delivery (F-1 mating) to produce the F2a and F2b generation and litters.

Male and female fertility was reduced in all treatment groups, including the controls for both the F2a and F2b matings. Fertility performance in each group was generally similar. Please refer to tables below.

First F-1 Mating Producing the F-2a litter

Fertility Index

Dose	Females: Pregnancies / Matings	%	Males: Fertile Males / Total Males Mated	%
0	12/25	48.0	11/25	44.0
20	12/25	48.0	12/25	48.0
100	13/25	52.0	10/25	40.0
500	15/24	62.5	14/25	56.0
Second F-1 Mating Producing the F-2b litter				
0	12/25	48.0	12/25	48.0
20	10/25	40.0	10/25	40.0
100	12/25	48.0	10/25	40.0
500	14/25	58.3	11/25	44.0

Results for F-1 females that did not deliver an F-2a or F-2b litter in the control, 20, 100 or 500 ppm treatment groups and did not exhibit a normal four- to five day estrous cycle when examined for 14 days after completion of the F-2b delivery, were as follows:

Dose (ppm)	0	20	100	500
No. not delivering	11	9	9	8
No. not exhibiting a normal estrous cycle.	8	9	7	6

Estrous cycle examinations were also performed on 2, 6, 4 and 2 females in the 0, 20, 100 and 500 ppm groups, respectively, that did deliver F-2a litters but did not deliver F-2b litters. No treatment-related differences were observed in the estrous cycles of these females.

Uterine examinations of each nondelivering female yielded no significant findings.

Sperm evaluations conducted on the F-1 males at sacrifice revealed reduced motility at the following dose levels:

Dose (ppm)	0	20	100	500
Reduced Motility	5	5	2	3

Parturition: F-0 mating (doses 0-1000 ppm) to produce the F-1 generation. F-1 matings (doses 0-500 ppm) to produce F-2a and F-2b litters.

There was no observable effect of treatment on the length of gestation or on the process of parturition for any dose level at any mating period.

Litter Parameters: Pup Viability. Mean Number of Viable Pups Per Litter. Generation/Litter

Dose (ppm)	F-1	F-2a	F-2b
0	11.0	13.3	11.9
20	12.6	11.7	10.2
100	11.4	13.0	12.2
250	13.3	-	-
500	12.7	10.8	12.1
1000	9.5	-	-

The mean number of viable pups per litter on lactation day 0 was slightly reduced in the 1000 ppm dosage group when compared to the control group for the F-1 generation.

The mean number of viable pups on day 0 for the F-2a generation/litter was slightly decreased in the 500 ppm group. This decrease was not, however, observed in the F-2b litter.

Pup Survival to Weaning: F-1 Generation. Statistically significant decreases ($p < 0.01$) in pup survival occurred in the 500 ppm dosage group on lactation day 4 and in the 1000 ppm dosage group on lactation days 4 and 7. Pup survival in the other dosage groups was comparable to the controls at all intervals. The F-2a and F-2b litters showed no statistically significant effects of treatment on pup survival at any of the intervals when compared to the controls.

Pup Weight at Birth: F-1 Generation. Pup body weights at birth in the 500 and 1000 ppm groups were statistically significantly decreased ($p < 0.01$, $p < 0.05$ respectively) when compared to the control values. Pups at lower dose levels were comparable to the controls at birth. Body weights of pups from the F-2a and F-2b litters of treated dams were comparable to the respective control groups at birth.

Pup Growth During Lactation: F-1 Generation. Mean body weights decreased slightly with increased dosage in males and females on lactation day 4 before and after reduction in all treatment groups with statistically significant ($p < 0.01$) decreases in the 500 and 1000 ppm groups. Mean body weight decreases were statistically significant for days 7, 14 and 21 at dose levels of 250 ppm and higher. F-2a Generation: Group mean body weight decreases were statistically significantly lower ($p < 0.01$) only on laccation day 7 at 500 ppm. All other decreases at this level were nonsignificant statistically. However, at 21 days and 500 ppm, both males and females showed weight losses of 14%. Values at 20 and 100 ppm were comparable to controls. F-2b Generation: Statistically significant body weight decreases in males and females were only observed on day 21 of lactation at a dose level of 500 ppm. However, on days 7 and 14, mean body weights were decreased 10% and 11%, respectively. All other values were generally comparable to controls.

General Observations: There were no notable findings with regard to general behavior or appearance in pups from any of the treatment groups through weaning.

Necropsy: Necropsy findings for F-1, F-2a and F-2b pups dying after lactation day 4 were not remarkable.

Gross Pathology:

F-0 Parents: It was reported that there were compound-related ulcerative (sores), necrotic or scabbing macroscopic changes observed among males and females receiving 1000 ppm and females receiving 500 ppm. This reviewer also notes that three male animals manifested necrotic skin at a dose level of 250 ppm. It was the opinion of the contractor that the additional changes observed were incidental and unrelated to compound administration.

F-1 Pups: There were no clear compound-related changes observed. However, changes suggestive of hemorrhage were observed in the stomach and small intestine of F-1 pups receiving 1000 ppm and dying before and after the parental selection. Red or black mucoid contents or red/black foci were observed primarily among animals receiving 1000 ppm of compound and dying after selection of F-1 parents. It was reported that male and female pups (5/sex/group) selected for necropsy and histological evaluation were free of macroscopic changes of consequence.

F-1 Parents: There were compound-related macroscopic changes observed in the skin of male and female F-1 parents killed at the terminal sacrifice. The open sores, hair loss and scabbing observed during the course of the study were also seen during necropsy, although with much lower frequency (and perhaps not in the same animal; reviewers note). Observations were noted primarily in males at 500 ppm. No apparent effects were observed at the lower dose levels. A moderately enlarged spleen and lymph node were also observed at 500 ppm in male. Other observations noted at necropsy did not appear to be compound-related.

Females showed some mild but generalized hair loss at 100 and 500 ppm. One female at 100 ppm did show scabbing of the head.

F-2a Pups and F-2b Pups: There were no compound-related macroscopic changes observed among male and female pups.

Microscopic Pathology: F-1 pups: Examination of the reported data indicated that there were no compound-related microscopic changes observed among F-1 male and female pups randomly selected for histopathological evaluation and sacrificed after selection of the F-1 parental animals. F-1 parents: There were no compound-related microscopic changes observed among male and female F-1 parents dying during the course of the study (2 males and 1 female at 500 ppm) or killed at a scheduled sacrifice. This reviewer finds no reason to disagree with this assessment. Five neoplastic lesions were observed among males and females receiving 0, 20 and 500 ppm. They were as follows:

0 ppm - male; C-cell adenoma

20 ppm - female; mammary fibroadenoma
male; myxosarcoma

500 ppm - male; malignant lymphoma
male; pituitary adenoma

F-2a pups: Examination of the reported data did not reveal any compound-related microscopic changes observed among male and female pups randomly selected for histological evaluation and sacrificed at weaning. The few microscopic changes observed were considered incidental and unrelated to the compound.

Organ weights: F-0 Males: Organ-to-body weight ratios revealed statistically significant increases for testes and adrenal at 1000 ppm. Females showed statistically significant organ-to-body-weight increases for spleen, liver, kidney, adrenal and thyroid gland at 1000 ppm. Thyroid was also statistically significantly increased at 500 ppm. Organ-to-brain-weight ratio for females showed a statistically significant decrease for heart at 500 and 1000 ppm, and a statistically significant increase for thyroid at 500 ppm. Organ-to-brain-weight ratios for males were not taken.

F-1 pups: Organ-to-body-weight ratios for males were statistically significantly decreased for spleen at 250 ppm. Statistically significant increases were noted at 500 and 1000 ppm for both brain and adrenal. Statistically significant increases were observed for females for thyroid at 250, 500 and 1000 ppm. The adrenal gland was also statistically significantly increased at 1000 ppm. Statistically significant decreases were noted for spleen at 500 and 1000 ppm and for liver at 500 ppm. Organ-to-brain-weight ratios were statistically significantly decreased for males and females at 500 and 1000 ppm for spleen liver, kidney and heart. The spleen was also statistically significantly decreased in males at 250 ppm. F-1 parents: Males and females both showed statistically significant decreases of organ-to-brain-weight ratios for thyroid, males only at 20 ppm and females only at 1000 ppm. F-2a and F-2b generation: Statistically signficant random changes in organ-to-brain-weight ratios were recorded for both males and females.

Discussion and Conclusion: Treatment with ZR-3210 Technical resulted in skin lesions of the parental rats at levels of 100 ppm and above over both generations. Lesions consisted of open sores, scabbing and hair loss and occurred in a dose-related fashion among animals of both sexes receiving 100 ppm or more of test article in the feed. They were observed on the head, neck, shoulders, fore-limbs and ears of the animals. In the F-0 animals, they occurred during study week 12 and continued throughout the generation, while in the F-1 parents they occurred starting in the 10 weeks after weaning, and some were seen throughout the generation. Variable degrees of healing took place. These lesions have been observed in other studies and are currently thought to represent an irritation associated with dermal contact of the test article in the diet. However, this has not been established with certainty. Animals receiving 20 ppm were not observed as having skin lesions.

Matting of the haircoat among females, but not males, of the F-0 generation was noted. The significance of this observation is not known.

Both sexes of these groups also exhibited a transitory condition of swollen, dry or cloudy eyes, which was inconsistently related to dosage level. The number of observations for this condition in animals of both sexes was not biologically meaningful at any dosage level during the following study weeks. Cloudy eyes were also seen in one animal of the F-1 generation.

Swelling of the ventral neck area was also observed in a few males and females in all groups, including controls, of the F-1 generation. This condition was temporary, lasting 6 weeks (weeks 54-60) and was not considered an effect of treatment. A viral infection was suspected as a cause of the swelling.

Mortality among F-0 animals was one each in the control and high-dose groups. In the F-1 generation, 33 animals in the 1000 ppm dose group died and 2 animals in the 500 ppm dose group died. Survival was 100% in both generations at dose levels of 20, 100 and 250 ppm.

Necropsy and histopathological findings for the most part were not significant and treatment-related alterations in organ weights among parental animals of either generation were not observed. However, hair loss, scabs, and open sores were observed at necropsy as they were during the in-life portion of the experiment. Some mandibular and regional lymph nodes draining the upper body were enlarged and most likely are a defensive response to the occurrence of the skin lesions. The presence of the skin lesions and enlarged lymph nodes were observed at a dose level beginning at 250 ppm. (Note here that during the in-life portion of the experiment, skin lesions were observed at dose levels beginning at 100 ppm.)

Treatment with ZR-3210 Technical has an inhibitory effect on body weight gain of parental animals at high dosages. In the F-0 animals, slightly reduced body weight gains were recorded for males of the 250, 500 and 1000 ppm groups, while only females of the 1000 ppm group had decreased body weight gains. These reduced values attained statistical significance at weeks 15 (males and females), 20 (males) and 24 (females). The females in the 500 and 1000 ppm groups also had decreased body weight gains on gestation days 15-20 and on lactation days 0-7, but control values were erratic at the latter time interval and the reduced values may lack biological significance.

In the F-1 generation animals, treated male body weights were generally less than the controls at all time intervals, but only at dosages of 250 and greater were such differences statistically significant. The females of this generation also had reduced body weights and these were apparent at dosages of 100 ppm and higher; however, statistical significance was reached only on week 25 for dose levels of 100 ppm and up. Maternal body weights were also decreased in the 100 and 500 ppm groups (the 250 and 1000 ppm groups having been sacrificed earlier) during gestation and lactation of the first litter; they were comparable to controls during the same period of the second litter and thus probably do not represent a significant finding.

Treatment with ZR-3210 Technical had little effect on food intake of test animals except at the highest levels. Compared to control values, F-0 males had slightly decreased food consumption over the generation at the 500 and 1000 ppm level while the F-0 females were affected only at the 1000 ppm level. This effect at the 1000 ppm level may be due to reduced palatability of the test diet. A palatability problem has been shown to exist with this compound in previous studies. This continued in the latter during lactation of their litters but, suprisingly, not during gestation. Among the F-1 animals, males at the highest dose level (500 ppm) had slightly reduced food consumption over the generation, while treated females had food intake essentially comparable to that of the controls.

Matings for the F-0 generation resulted in reduced fertility for a number of the treated groups. Fertility rates of the control, 100 and 1000 ppm groups were comparable. These matings consisted of cohabitation of the dams with a single male for 15 days; evidence of insemination was determined by the presence of a copulatory plug in the female. A number of nondelivering dams from all treatment and control groups had evidence of insemination; some others delivered with no evidence of insemination. Uterine examination of the dams not delivering did not demonstrate any consistent significant findings. Sperm evaluation from a number of males from both treated and control groups demonstrated a few animals with reduced sperm motility or immotile sperm; however, a reexamination of their earlier breeding performance indicated that a number of these males successfully impregnated dams in spite of the sperm findings. There also were no consistent findings upon testicular examination.

The cause, then, of the reduced fertility among some of the treated groups in the F-0 mating remains a puzzle, although it appears that it cannot be ascribed to the treatment *per se*, since acceptable (i.e., comparable to control) fertility indices were observed in the 100 ppm and the 1000 ppm dose levels. The fact that a number of the presumed inseminated dams did not come to delivery and that some delivered in the absence of signs of insemination suggests technical error, at least in part, in evaluating the insemination process.

The F-1 matings to produce the second generation were also less than anticipated. The first mating of the resulting litters (F-2a) consisted of cohabitation with the female by one of three different males as required to achieve insemination, in intervals of 10, 5 and 5 days. Insemination was again determined by the presence of a copulatory plug. The overall fertility rates were 48, 48, 52 and 62% for the control, 20, 100 and 500 ppm groups, respectively. That these low percentages were not totally attributable to the dams is shown by the fact that the respective rates were virtually identical when caluclated on the basis of male fertility (fertile males/total males mated); these were 44, 48, 40 and 56%.

At this point, it was decided to breed for still another litter in order to obtain more data on this apparent problem and attain the prescribed number of litters from the F-1 dams. This mating to produce the F-2b generation again consisted of cohabitation by dams with up to 3 different males at 10, 5 and 5 day intervals as necessary to obtain insemination. Reliance was placed on the presence of vaginal sperm rather than copulatory plugs. The fertility

indices were virtually identical to those obtained in the first mating: 48, 40, 48 and 58%, respectively, for control, 20, 100 and 500 ppm. Male fertility indices were 48, 40, 40 and 44% for the same groups. A majority of the females not delivering either an F-2a or F-2b litter did not exhibit a normal 4-5 day estrous cycle, but some did, and there were no significant uterine findings relating to infertility. Sperm evaluations of the F-1 males demonstrated reduced motility in some animals, but, as in the F-0 sperm evaluations, some of these successfully inseminated females (i.e., females that delivered), and thus the biological finding is open to question.

It appears that the reduced fertility over both generations is not a treatment - (i.e., compound) related effect, since control fertility indices in all three matings were equivalent to those of the treated groups. Some of the infertility findings resulted from technical misjudgment on the part of the technician; however, this does not alter the fact that the parental animals in both generations did not possess the ability to conceive as expected. The inability to conceive as expected was apparently not related to age at breeding, environmental factors during cohabitation, potentially associated gonadal pathology or any other factors (as stated by the contractor) to this finding. Whatever the etiology, both sexes were affected, and there was no apparent association to treatment.

Treatment with ZR-3210 Technical has no demonstrable effect on length of gestation or on the parturition process in either generation.

Pup viability at birth and survival to weaning were affected by treatment in the first generation. For the F-1 pups, the mean viable pups per litter at birth was slightly reduced at the 1000 ppm level, while statistically significant decreases in pup survival during lactation were recorded at the 500 and 1000 ppm levels. The mean number of viable pups/litter at birth for the F-2a and F-2b litters were generally comparable to controls. Pup survival to weaning was not affected by treatment in either litter in the second generation.

Pup weight at birth was reduced in the first generation at the 500 and 1000 ppm levels, but was comparable to controls among both litters of the second generation.

Pup growth during lactation was inhibited in the first generation at the higher dosage levels. This inhibition of growth was manifested on lactation day 4 in the 500 and 1000 ppm groups and on lactation days 7, 14 and 21 at dosages of 250 ppm and higher. Growth of pups was also reduced in both litters of the second generation at a dose of 500 ppm. Pup body weights in the 100 ppm group were reduced in the second generation, while those of pups in the 20 ppm group were comparable to or exceeded those of controls at all lactation intervals in both litters.

There were no significant treatment-associated findings with respect to appearance or behavior in any of the pups of either generation. Necropsy and histopathological examinations of the pups failed to demonstrate any significant treatment-related findings.

Based on the results of the experiment, treatment with ZR-3210 Technical at a dosage level of 20 ppm in the diet was concluded to be the clearly demonstrated "no-effect" level for the study.

Classification: Core-Guideline.

Fetal

20 ppm = NOEL

100 ppm = LEL = pup (weight) growth slightly reduced

250 ppm = significantly decreased pup (weight) growth

500 ppm = significantly decreased pup weight at birth, survival to weaning and pup growth.

1000 ppm = significantly decreased pup weight at birth, survival to weaning, and pup growth. Pup viability slightly reduced at birth. Death of 33 offspring, 25 to 32 days of age

Parents and Adults

20 ppm = NOEL

100 ppm = LEL = skin lesions 100 ppm and higher (both generations), decreased body weight (females), maternal body weight decrease during gestation and lactation for F-2a generation only.

250 ppm = reduced body weight gain (males), decreased body weight for males and females.

500 ppm = reduced body weight gain (males), body weight decrease in males and females, maternal body weight decrease during gestation and lactation for F-2a generation only.

1000 ppm = reduced body weight gain in males and females, maternal body weight decreases during gestation and lactation, body weight decreases in males and females.

Reproduction Study: The following question(s) have arisen in the course of the review:

1. The Toxicology Branch does not disagree with the conclusion that the NOEL for the study is 20 ppm. However, we would ask the petitioner to submit a line of reasoning which separates those effects for the study which have led to a NOEL for the study, from those effects which have led to a NOEL for reproductive effects per se.

2. Identify the strain of rat used in this study.

HED:KOCIALSKI/BAILEY:DCR-24271:WANG-0055A:RAVEN:479-2013:7/27/82

Subject: Viral Screening Study Carried-Out in Conjunction With The Rat Two Generation Reproduction Study

Accession No: 070661 filed in conjunction with Accession No. 070660

Testing Facility: Microbiological Associates
Bethesda, Maryland

IRDC Study Number: 322-041 filed in conjunction with IRDC Study No. 322-039

Report Submitted to Sponsor: September 5, 1980

Objective: To evaluate viral exposure of rats received from the Charles River Breeding Laboratories, Inc., Portage, Michiga upon receipt at IRDC and after 17 weeks in-house.

Procedure: A detailed experimental protocol can be found in the above referenced accession.

Immunological tests to detect the presence of the following eleven viruses were performed. They were identified as follows:

Reovirus type 3 (Reo III)
Pneumonia virus of mice (PVM)
Encephalomyelitis (GDV II)
Sendai (Send)
Mouse adenovirus (M. Ad.)
Sialodacryadenitis (SDA)
Mouse hepatites (MHV)
Toolan H-1 (H-1)
Kilham rat virus (KRV)
Rat coronavirus (RCV)
Lymphocytic choriomeningitis (LCM)

Results:

Phase 1 results reflect those viruses identified in rats which were examined within 24 hours of receipt at IRDC.

Positive results were obtained for only Kilham Rat Virus (KRV).

Phase 2 results reflect those viruses identified after 15 weeks on study (17 weeks in-house). Positive results were obtained for the following viruses:

Pneumonia virus in mice
Kilham rat virus
Sendai
Rat coronavirus
Sialodacryadenitis

Two positive reactions to MHV were considered to be due to a cross reaction with other viral antibodies and not a true indication of Mouse Hepatites (MHV).

Discussion: It was reported that external observation produced no obvious symptoms of viral disease (such as corneal opacities or respiratory distress) in any of the animals on study. The Phase I viral screen indicated the presence of Kilham Rat Virus (KRV) in the Charles River colony in Portage, MIchigan. The Phase II rats showed KRV in about the same proportion as in Phase I. Most of them also showed moderate to high titres of pneumonia virus in mice, Sendai virus, rat caronavirus and sialodacryadenitis.

Contamination with these four additional viruses could have taken place in 1) the original Charles River Labs 2) in the transport between supplier and IRDC or 3) at IRDC. If they were contaminated prior to arrival at IRDC, their titres of antibodies against each virus would not be high enough to detect for at least 2 weeks subsequent to exposure. Therefore, it was impossible to determine precisely where the viruses were introduced.

Classification: Supplementary

NOTE: Personal communication between Norma Jean Galiher (Zoecon Corp.) and Dr. A. Carl Kahn III (Director of Clinical Pathology, IRDC) apparently indicates thay the five viruses for which the animals showed titres are not in and of themselves directly responsible for the appearance of abnormal epidermal conditions (other than keratitis).

#ml4

Subject: 14-Day Range Finding Study in CD-1 Mice. Final Report.

Test Compound: Fluvalinate (Mavrik®)
Technical Material (1) ZR-3210
Half-Resolved Technical (2) ZR-3210 Racemic Mixture

Accession No: 070661

Testing Facility: Litton Bionetics Inc.
Kensington, Maryland

Study Number: LBI Project No. 22070

Responsible Professionals:

David R. Damske - Toxicology Group Leader
Francis J. Mecler - Study Director
Douglas K. Craig - Director, Department of Toxicology

Testing Period: November 7 - November 21, 1981

Report Submitted to Sponsor: January 1981

Revised Report Submitted to Sponsor: April 1981

Batch or Lot Number and Purity of Sample:

ZR-3210 Half-Resolved Fluvalinate (445-95) #1080-91; Purity of 89.9%

ZR-3210 Racemic Technical Run #7, Anal. No. 0979-069; Purity of 93.8%

Homogeneity, Stability and Concentration in Feed: Fluvalinate can be accurately formulated at concentrations between 5 and 1000 ppm and homogeneously distributed in animal feed. Diets were shown to be stable up to 40 days at room temperature. The analytical method has good precision and a good sensitivity range.

Objective: The purpcses of this study were to compare the toxicity of the test materials of two different isomeric compositions and to determine dosage levels to be used in a mg/kg subchronic feeding study with one of these materials.

Materials and Methods:

Seventy (70) male and 75 female mice of the CD-1 strain were obtained from the Charles River Breeding Laboratories, Portage, Michigan and acclimated for two days at the test facility. The mice were acclimated and housed in a temperature controlled animal room. Artificial lighting was provided on a 12 hour light/dark cycle. Feed and water were provided ad libitum. Individual animal numbers were assigned and the mice were identified by toe clip, ear punch and cage tape. The mice were 38 days old and weighed from 19.6 to 30.6 grams at the time of the first presentation of the treated diet. The formulations were prepared so as to correct for the purity of the test materials. No attempt was made to adjust the diet concentrations for changes in body weight and food consumption during the 14 days of the study. The basal diet used in this study was Purina Certified Laboratory Chow® 5002. Reagent grade acetone was used as the vehicle. The mice were weighed before being fed the treated diets and after 7 and 14 days of treatment. Food intake was determined at 7 and 14 days and fresh diets were placed in the cages at 7 days. The animals were observed daily for signs of toxicity and were checked twice a day for death. Mice found dead were subjected to immediate gross necropsy. After 14 days of observation surviving mice were sacrificed using chloroform and subjected to a complete gross necorpsy.

Mice were divided into 13 treatment groups of 5 males and 5 females by use of a computer generated random number table. Extra animals were discarded from the study. The 13 treatment groups were as noted in the table below.

Technical Fluvalinate Half-Resolved

Group	Dose Level - mg/kg/day	- ppm
1	Acetone Control	
2	0.03	0.2
3	0.10	0.7
4	0.30	2.0
5	1.00	7.0
6	3.00	20.0
7	10.00	70.0
8	30.00	200.0
9	100.00	700.0

Technical Fluvalinate Racemic

Group	Dose Level - mg/kg/day	- ppm
10	3.0	20.0
11	10.0	70.0
12	30.0	200.0
13	100.0	700.0

Results: Observations:

All animals were observed for the following parameters:

- ataxia
  reduced respiration
  body temperature (by touch)
  arched back
  excessive salivation
  immobility
  prostrate in cage
  reduced motor activity
  tremors
  rough hair coat
  peri-anal yellow stains
  thinness
  death
- local hair loss
  local crusting
  skin paleness
  ulcerations

Half-Resolved Technical - Males and Females:

Dose: 100 mg/kg/day:

Signs observed in 4 or more females were:

ataxia
excessive salivation
reduced motor activity
skin paleness
death

Signs observed in 3 or more females were:

local hair loss (only sign)

Signs observed in all other categories were generally represented by one (1) female each.

Signs observed in 4 or more males were:

excessive salivation
skin paleness
rough hair coat

Signs observed in 3 or more males were:

local hair loss
local crusting

Signs observed in categories represented by at least one (1) animal each were:

ataxia
cold to touch
arched back
reduced motor activity
peri-anal yellow stains
death

Dose: 30 mg/kg/day:

Signs at 30 mg/kg/day for both males and females were nearly exclusively confined to the following categories:

local hair loss
local crusting
ulcerations

Dose: 10 mg/kg/day:

Signs observed in one (1) female each were noted for the following:

local hair loss
local crusting
ulceration

No signs were observed for males at this or lower dose levels.

No signs were observed for females at dose levels less than 10 mg/kg/day.

Racemic Technical - Males and Females:

Signs observed in females at 10, 30 and 100 mg/kg/day were primary confined to the skin and were manifested as,

local hair loss
local crusting
ulcerations

Signs were observed at 10 mg/kg/day (local hair loss) which then progressed to the other categories of local crusting and ulcerations and involved an increasing number of animals as the dose was increased.

Reduced motor activity and rough hair coat were observed only at the high dose level.

The only affects noted in males occurred at the high dose. Each of 2 animals were affected in the following categories:

local hair loss
local crusting
rough hair coat

Results: Body Weight/Daily Food Intake:

Half-Resolved Technical - Males and Females:

Males at the highest dose level manifested a statistically significant ($p < 0.05$) body weight decrease at 7 and 14 days when compared to the control group. Daily food intake was not statistically significantly different from control values at any time.

Females at the highest dose level manifested a statistically significant ($p < 0.05$) body weight decrease at 7 days. Daily food intake was comparable at this period. Four of five animals were found dead at 14 days which precluded any meaningful measurement for the high dose group.

Racemic Technical - Male and Female:

Male and female body weights and daily food intake were comparable to control values at all dose levels.

Results: Body Weight Change:

Half-Resolved Technical - Male and Female:

No statistically meaningful differences were observed from control values for males and females up to and including 10.0 mg/kg/day.

A statistically significant decrease was noted for females on day 14 in the 30 mg/kg/day dose group. Four of five females died at this time period at the next highest dose. Males and females showed statistically significant decreases at the highest dose levels.

Racemic Technical - Male and Female:

A statistically significant decrease was observed in females only at 14 days in the 100.0 mg/kg/day dose group.

Pathology: It was reported that gross necropsy of mice which died on study revealed no changes not commonly seen in mice which are found dead. No visible abnormalities were seen in the animals killed at day 14.

Discussion: The results indicate that on a mg/kg basis the Half-Resolved mixture is much more toxic than the Racemic mixture. Additionally females appear to be more sensitive to the toxic effects of compound than are males. This is seen by the greater number of parameters and larger number of animals showing signs. This conclusion is also supported by the statistically significant decreases in body weights and body weight changes.

Signs of hair loss, crusting and ulceration were localized to the head, forepaws and shoulder areas of the mice and were primarily observed in the high dose groups. A dose response was not observed with the half-resolved mixture with responses being similar at 30 and 100 mg/kg/day. However an increased response with a higher dose was observed at 100 mg/kg/day in males and females receiving the racemic mixture when compared to the next lower dose.

Minute examination of the skin and fur revealed aggregations of feed particles deep in the fur of the animals. It was postulated that the local hair loss and skin lesions might be due to the prolonged contact of the test material matted in the fur of the mice.

Actual compound intake was calculated from the concentration of test material in diet, the body weight and food intake. The actual intake ranged from 1.5 to 2.0 times the intended compound intake. This difference occurred due to the difference between the assumed food intake used to calculate the concentration and the actual food consumed.

Conclusions:

Half-Resolved Technical is more toxic on a mg/kg basis than is the Racemic Technical. Females appear to be more sensitive than males. Quantitative differences in observations can be attributed to compound potency. There do not appear to be any qualitative differences between the two mixtures.

Classification: Supplementary.

#m14

Subject: 90-Day (13-Week) Dietary Toxicity Study In Mice. Final Report. 002256

Test Compound: Fluvalinate Technical (Half-Resolved)

ZR-3210 Technical (Half-Resolved)

Mavrik(R) Technical (Half-Resolved)

Accession No: 070663

Testing Facility: Litton Bionetics, Inc., Kensington, Maryland

Study Number: LBI Project No. 22088

Responsible Professionals:

Group Leader - D. Maloney

Senior Toxicologist - R. J. Weir

Study Director - L. A. Goldsmith

Pathologist (at necropsy) - Dr. Marion Valerio, D.V.M.

Pathologist (histopathological evaluation) - Dr. Richard Montali, D.V.M.

Testing Period: March 23, 1981 - June 23-25, 1981.

Report Submitted to Sponsor: November, 1981.

Purity of Test Material, Batch and Lot Number

Half-Resolved ZR-3210 Technical (fluvalinate) 89.9%, Analytical No. 10801-91.

Half-Resolved ZR-3210 Technical (fluvalinate) Run 23R Anal. No. 0281037; 93.1% pure. Note here that this material (i.e. Anal. No. 0281037) was used for the diet mix of June 10, 1981 which was the last diet mix for the study. This was additional test material required to complete the study.

Homogeneity, Stability and Concentration in Feed: The stability of the test material in the diet and the ability of the proposed diet formulation procedure to produce a homogeneous concentration of test material in the diet was verified prior to dosing. Diets were homogeneous and stable for at least 14 days under ambient conditions.

Materials and Methods: One hundred and seventy-five (175) weanling CD-1 mice were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan. The animals were acclimated for 12 days to laboratory conditions in the room in which the study was to be conducted. The animals were inspected by a veterinarian and good health verified for all animals to be placed on test. During the acclimation period, the animals were randomly assigned to five (5) test groups and one (1) control group each consisting of 10 animals per sex per group according to a stratified randomization method. The animals in each group were weighed and the group mean body weights were analyzed to assure that no statistically significant differences existed. The animals were identified by toe clips, ear punches and cage cards.

The animals were individually housed in hanging stainless steel cages which were changed and sanitized weekly. No other chemical or animal species were under concurrent investigation in the room in which the study was conducted. The environmentally controlled room provided 12-15 changes of

fresh air every hour, a 12 hour light/dark cycle, controlled temperature (23°C ± 2°) and humidity (40-70% RH). No significant deviations from the prescribed environmental conditions occurred during the study.

The animals had free access to tap water contained in glass water bottles. The water bottles were changed weekly. The water was acidified to ph 2.5 ± 0.2 with hydrochloric acid. The acidified tap water (water was acidified to control for psuedomonas, a common bacteria found in water) was quantitatively analyzed for the following contaminants: lead, mercury, cadmium, arsenic, barium, chromium, selenium, lindane, silver, fluoride, gross alpha and beta particles, methoxychlor, nitrate, silvex, toxaphene, 2,4-D and endrin (note here that the results were all well below the maximum tolerated limits established by E.P.A.). Results are available upon request.

Test diets were prepared fresh weekly using acetone as a vehicle with the test material administered as part of the basal diet (Purina Certified Rodent Chow(R) #5002) to each sex group of each of the treatment groups on a mg/kg/day basis. The appropriate concentration was based on the previous week's body weight and food consumption values for that group. Values for the first part of the study were obtained during the acclimation period. The animals were supplied with a measured amount of feed in the special feeders (Wahmann LC 207/A glass feeding jars) to prevent excessive feed spillage.

The animals were 41 days old on the first day of dosing. Males weighed 17.8 to 28.6 grams and the females weighed 14.5 to 24.5 grams. Ten males and 10 females comprised each of the following dose groups.

Group No.	Dose (mg/kg/day)
1	0.0
2	1.0
3	3.0
4	30.0
5	50.0
6	100.0

All animals were observed twice per day (at least 6 hours apart), 7 days per week for appearance, abnormal behavior, toxic signs, moribund condition or mortality. Each animal was removed from its individual cage and identified, weighed and given a physical examination, including palpation, initially and weekly for 13 weeks. Food consumption was determined over a 4 day period each week. Clinical observations, body weights, and food consumption data were entered directly into a HP 1000 computer by toxicology technicians via portable computer terminals inside the animal rooms. Any animal judged to have been moribund was sacrificed using carbon dioxide and subjected to the same post-mortum exam received by animals found dead or killed at termination.

The study was terminated after 13 weeks of treatment. The animals were fasted overnight and sacrificed via overexposure to carbon dioxide. Just prior to sacrifice, all surviving animals judged capable of surviving the procedure from the control 3.0, 30.0, 50.0 and 100.0 mg/kg/day groups were bled via orbital sinus puncture. The following tests were performed:

hematocrit (HCT)	total leukocyte count (WBC)
hemoglobin (HGB)	differential leukocyte count
erythrocyte count (RBC)	reticulocyte count (RET)

A complete gross necropsy was performed on all animals killed at termination under the supervision of a board certified veterinary pathologist.

The following organs were weighed for all animals:

brain	uterus
heart	kidneys[1/]
liver	ovaries/testes[1/]

([1/] These organs were weighed as a pair unless one organ was grossly abnormal.)

Organ weight/body weight and organ weight/brain weight ratios were calculated.

The following tissues or parts thereof were taken from all animals found dead, moribund, sacrificed or terminally sacrificed and preserved in 10% buffered formalin for future histopathologic evaluation if required. The tissues from all control and high dose animals were examined microscopically by Dr. Valario. Dr. Valario reviewed the gross necropsy findings with Dr. Montali prior to histopathological evaluation.

The following tissues were examined:

All gross lesions and tissue masses

Brain (cerebrum, cerebellum, brainstem)

Heart

Spinal Cord (cervical, thoracic and lumbar)

Sciatic Nerve (distal section)

Lungs (with main stem bronchi)

Liver (including gall bladder)

Ovaries

Testes

Uterus (corpus, cervix)

Adrenal Glands

Kidneys

Statistical analysis using Dunnett's t-test ($p < 0.05$) was conducted on mean body weight, food consumption, organ weight and hematology data (when appropriate).

Results: Skin Lesions: Males and Females:

The most commonly reported clinical finding in the course of the 13 week feeding study was the presence of small (<1.0 cm) scabs and alopecia. The pattern of distribution and the high incidence of these lesions on the head, neck and shoulders suggested contact irritation with the medicated feed as the probable cause of the lesions; however, a systemic efect could not be ruled out. None of the control animals of either sex exhibited skin lesions throughout the study, whereas lesions were seen with increased frequency on

the higher dose groups. The clinical appearance and progression of these lesions were typified by local alopecia and erythema which was often associated with what appeared to be oozing of serous fluid. These lesions would progress through a course of slow healing. Eventually, normal appearing skin was present at the site of the original lesion. Often, a similar lesion developed in an area adjacent to a healing lesion. Incidences of similar lesions did occur in the 1 and 3 mg/kg/day males and in a single animal of the 1.0 mg/kg/day female group.

Tabular data of male animals showing at least one scab indicated that the cumulative total for all dose groups on a weekly basis remained relatively constant (range of 22-27) after the second week through completion of the experiment. Additionally, there was no increase with time within dose groups after 3 weeks through termination. The number of males showing at least one scab at any one weekly period during the course of the study (plus or minus one animal) were generally as follows:

Dose (mg/kg/day)	Response
0.0	0
1.0	1
3.0	1
30.0	6
50.0	9
100.0	10

The cumulative total numbers of observations (scabs) recorded at the conclusion of the experiment for males at each dose level was as follows:

8.

Dose (mg/kg/day)	Response
0.0	0
1.0	3
3.0	18
30.0	69
50.0	109
100.0	114

Inspection of the above table demonstrates that although increased responses were observed with increased dose, there was no log-dose response increase from 50 to 100 mg/kg/day with the values suggesting that a maximum response had been attained at 50 mg/kg/day.

Additionally, an analysis of the individual animals manifesting scabs, at the arbitrarily chosen time periods of 3, 6, 9 and 12 weeks by this reviewer, does confirm a random pattern of appearance, disappearance and reappearance of scabs in some animals, as well as the continued presence of a scab in many animals for all four (4) arbitrarily chosen time periods.

Females: Generally speaking, the results for females vary in only a minor way from the results reported for males. A parallelism of results appears evident. The points and rationale presented by this reviewer for males are equally applicable to the females.

The cumulative total number of observations (scabs) recorded at the conclusion of the experiment for females at each dose level was as follows:

Dose (mg/kg/day)	Response
0.0	0
1.0	5
3.0	0
30.0	48
50.0	94
100.0	106

It is pointed out here that in contrast to the 18 responses (scabs) recorded for males at 3.0 mg/kg/day, females recorded zero responses at the same dose level. This particular difference does not challange this reviewer's conclusion of the parallelism of results between the sexes and the rationale presented.

No masses were palpated on any of the animals throughout the course of the study and none were observed during gross necropsy. No tumors were discovered in the control or high dose groups when tissues were examined histologically.

Survival was 70% or better in all groups with 9 of the 12 sex groups showing a 90% or better survival rate. A log-dose response for animals dying intercurrently or sacrificed in a moribund condition was not observed. Two males and 2 females of the 30 mg/kg/day dose group were 4 of the 6 animals sacrificed in a moribund condition. The 2 others were both males with one each coming from the 50 and 100 mg/kg/day dose groups.

A statistical analysis of male body weights for each of the 13 weeks the compound was administered, revealed statistically significant body weight

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decreases and a log-dose response at dose levels of 30, 50 and 100 mg/kg/day when treated groups were compared to control groups. The body weights for the 1.0 and 3.0 mg/kg/day dose groups were consistently lower than controls, but were not statistically significant.

Analysis of female body weights revealed comparable values for all treated groups when compared statistically to the control group. However, the 50 and 100 mg/kg/day groups did have lower mean body weight values during the latter part of the study and might be considered a compound related effect.

Food consumption values for males at 1.0, 3.0, and 30.0 mg/kg/day were not statistically significantly different from control values and appeared to be generally comparable on a numerical basis. However, the 50 and 100 mg/kg/day dose groups manifested an increased food consumption, when compared to controls, on a consistent basis and on some occasions showed statistically significant increases ($p < 0.05$). Food consumption values were therefore considered to be equal to or greater than control values for treated male mice.

Food consumption values for females showed no statistical differences when treated groups were compared to the control group.

Hematological values for males were much lower, but not statistically significantly lower, than those recorded for controls at 50 and 100 mg/kg/day in the following categories: RBC count, hematocrit (HCT), and hemoglobin (HGB). The reticulocyte count (RET) was raised but not statistically significant at 50 and 100 mg/kg/day. The WBC count was substantially

increased at 50 and 100 mg/kg but the values were not statistically significant. The WBC (white blood cell) differential count showed very large but no statistically significant increases for banded and segmented neutrophils, lymphocytes and monocytes at 50 and 100 mg/kg dose levels.

Hematological values for females for RBC count, HCT and HGB were all statistically significantly lower ($p < 0.05$) than control values at a dose level of 100 mg/kg/day. Values for RBC, HCT and HGB were substantially lower than control values at 50 mg/kg/day with the HGB values being statistically significant at the $p < 0.05$ level. Reticulocyte counts were substantially above control values at 50 and 100 mg/kg/day, but were not statistically significant. The white blood cell (WBC) count was substantially higher than control values at 50 and 100 mg/kg/day with the value at a 100 mg/kg/day being statistically significant. The Schilling differential values for females were substantially but not significantly raised for banded and segmented neutrophils and monocytes at dose levels of 50 and 100 mg/kg/day.

The male body weights at the terminal kill showed statistically significant body weight decreases for dose levels of 30, 50 and 100 mg/kg/day. Absolute organ weight decreases were noted for kidney, liver, heart and testes at 100 mg/kg/day and for kidney at 50 mg/kg/day. All values were statistically significantly lower. Organ to body weight ratios were statistically significantly greater for brain and liver when compared to control values at 50 and 100 mg/kg/day. Organ to brain weight ratios showed no statistically significant differences at any dose level for any organ.

The female body weights at the time of terminal kill were not statistically significantly different at any dose level. However, body weights were 13% lower than control values at 50 and 100 mg/kg/day. The absolute organ weights of the ovaries were also statistically significantly lower than controls but only at the 100 mg/kg dose level. The organ to body weight ratio at 100 mg/kg/day was statistically significantly increased for liver and kidney and statistically significantly decreased for ovaries at a dose level of 100 mg/kg/day. Kidneys were also statistically significantly greater at 50 mg/kg/day. A statistically significant decrease in kidney weight was recorded at 3.0 mg/kg, but did not appear to have any biological implications. A statistically significant decrease was also observed for the liver at 1.0 mg/kg/day; this value was not considered to be biologically meaningful. The organ to brain weight ratios were all comparable with the exception of the ovary brain weight ratio which was statistically significantly lower from controls at 100 mg/kg/day.

Gross Pathology: There were skin lesions in the treated animals with the following incidence at the time of the terminal kill.

Dose (mg/kg/day)	Males	Females
Control	0	0
1.0	0	0
3.0	1	0
30.0	7	4
50.0	10	10
100.0	10	8

The lesions were oriented at the anterior ends of the mice and primarily affected the head, face, ears, neck and thoracic regions. The lesions were characterized as irregular, hairless, crusty, reddish-brown plaques that measured from several milliliters up to two centimeters across. The lesions were more numerous in the higher dosed animals and appeared to be more extensive in males. Many of the mice with skin lesions also showed enlargements of the mandibular lymph nodes and some showed enlarged axillary or prefemoral lymph nodes and splenomegaly. Several high-dose animals had opaque corneas. Other pertinent changes noted grossly were cystic ovaries in one animal each of the control group, the group receiving 50 mg/kg/day and 3 observations in the 30 mg/kg/day dose group.

Histopathology: Microscopically, the skin lesions in both male and female mice showed focal epidermal necrolysis with attendant acute inflammation. In areas adjacent to the necrosis there was acanthosis (epidermal hyperplasia) and follicular hyperplasia. The necrolysis had advanced to ulceration in some instances. There was also extensive acute and chronic inflammation characterized by marked dermal fibrosis in the dermis beneath the affected epidermal regions and ulcers.

Bacterial colonies were evident in some of the lesions superficially, but not below the epidermal layers. There was pseudoepitheliomatous hyperplasia evident in 2 high-dose females but clear cut changes of malignancy were not observed in any of the skin lesions.

Microscopic changes in the eyes, superficial lymph nodes and spleens were observed exclusively in treated mice with cutaneous lesions. Affected lymph

nodes showed follicular hyperplasia, predominantly of germinal centers, and a marked increase in the number of plasma cells in the medullary cords (plasmacytosis). The necrosis observed in the lymph nodes involved both lymphocytes and plasma cells.

The ocular changes were predominantly of an acute inflammatory nature with involvement of the corneas and intraocular chambers.

Splenic enlargements were also observed and confirmed the gross findings.

There was also an increased frequency of ovarian cysts in the high-dose group of females (2/10 in the control group and 5/9 in the high dose group). The cysts were typical of the parovarian type which are lined by low columnar to cuboidal cells, some of which are ciliated. Only one of the ovarian cysts in the control group was seen at gross necropsy, but none in the high dose group was visible at necropsy. Microscopically, most ovaries from the treated group, overall, appeared smaller and had fewer or no corpora lutea (hypoplasia, NOS).

There were several other types of microscopic changes in one or both groups which included usually slight degrees of inflammatory changes in the liver and the kidneys, slight metamorphosis in the liver, minor congestion in the adrenals and hemorrhage in the lungs. The hemorrhage and congestion were considered agonal. The other changes were each of a low or random incidence or were distributed evenly in both control and treated groups and were not attributed to the treatment with fluvalimate.

It was the opinion of the pathologist that the cutaneous changes were clearly associated with the administration of compound. Because of the focal nature and random distribution of the lesions around the head, face and neck, the cutaneous effect of the compound was considered to be a result of topical irritation. Although a systemic efect could not be ruled out, cutaneous lesions induced systemically would be expected to be more diffuse and at least symmetrical. The types of cutaneous lesions described were, in the opinion of the pathologist, compatible with those induced by irritating substances. The pathogenesis of the lesions once the skin contamination occurred could not be determined by the pathologist. pathologist's report indicated that the substance was not apparently an irritant once it was ingested as there were no clinical manifestations and no gross (no histological examination of the intestinal tract took place) changes in the digestive tract or other organs to indicate that possibility.

The bacterial colonies noted on the surfaces of some of the lesions were considered as a complication of the loss of integrity of the integument. Ocular lesions were also considered to be the result of topical irritation by the compound. Superficial lymph nodes affected were the regional nodes that drained the inflammed skin, hence, the proliferative changes observed were considered responses to the extensive cutaneous inflammatory reactions. Increased hematopoietic activity in the spleens was considered secondary to the heightened inflammatory responses in the affected mice.

Less clear in the mind of the pathologist was the nature of the ovarian changes in treated females, but the decreased amount of luteal tissue and

increased parovarian cyst formation was accompanied by a significant weight decrease ($p < 0.05$) of the ovaries as compared with the controls. The cysts were compatible with those described in mice as arising from the rete tubules or from embryonic structures. They are known to occur spontaneously in early life although they are more common and larger in aging mice. (Ref: Lemon, P.G., and Gubareva, A.V., Tumors of the Ovary, in Pathology of Tumors in Laboratory Animals, Volume II, Y.S. Turusov, Editor, pp. 386, IARC Sci. Public No. 23, 1979).

Discussion and Conclusion: The compound was found to be non-tumorigenic up to 13 weeks under the conditions of the study.

The primary clinical sign observed was lesions of the skin in treated animals. Skin lesions were not observed in control animals. Lesions were primarily observed in the upper half of the body of both males and females. The number of lesions increased with an increase in dose and appeared to be log-dose responsive up to 50 mg/kg/day. The highest dose of 100 mg/kg/day did produce an increase in the number of lesions observed at 50 mg/kg but was not log dose resporsive to the lower dose. Although a systemic cause of the lesions could not be ruled out, the fact that no log-dose response increase was observed at the higher dose levels and that a systemically induced cause for the lesions would be expected to produce a more diffuse and at least a symmetrical response in affected animals seems to argue for a topical irritant effect.

The lesions were observed at all dose levels, with the significance of the findings at the lowest dose levels (1.0 mg/kg/day) being strengthened by the

presence of the similar type lesions at the higher dose levels for both sexes.

Animal survival was excellent at 70% or better and reflects good experimental design and animal husbandry.

Body weight decreases were observed for males at all dose levels with the decreases at 30, 50 and 100 mg/kg/day being statistically significantly lower. Body weight decreases can be attributed to compound toxicity since food consumption was comparable to control values for 1, 3, and 30 mg/kg dose levels and greater than control values at 50 and 100 mg/kg/day. Food spillage was most likely not interpreted as increased food consumption since special feeder jars were used and one might expect that any food spillage which would occur most likely would be evenly distributed between all groups.

Body weight changes for females were statistically comparable to control values at all dose levels as was food comsumption. However, body weights taken just prior to the terminal kill showed a 13% decline at both dose levels of 50 and 100 mg/kg/day.

Hematological effects were generally similar for males and females at dose levels ranging from 50 to 100 mg/kg/day. Decreased hematocrit, hemoglobin and red blood cell count with an attendant rise in reticulocyte count were suggestive of an anemia. Whether or not the anemia was a direct toxic effect induced by compound or a secondary effect resulting from some other cause (lesions) was not determined. The rise in the white blood cell count was

If the skin lesions are disregarded, the 3.0 mg/kg/day dose group in males would appear to be the highest dose group which showed no effects other than skin lesions based on the parameters tested. Effects at 30 mg/kg/day (the next level above 3 mg/kg/day which was tested) which produced adverse effects were body weight decreases in males. This was the only adverse effect observed at this dose level for either sex. Blood parameters were affected in both sexes at 50 and 100 mg/kg/day.

Classification: Supplementary

The following questions were raised during the course of the review.

1. The protocol states that all gross lesions were to be examined microscopically. Why weren't the ovarian cysts which were observed grossly in the Group 3 and 4 females examined microscopically?

2. Did the animals give any indication of itching or scratching?

3. Were the lesions of sufficient size or character to affect the blood parameters?

HED/TOX:KOCIOLSKI/BAILEY:DCR-24864:WANG-0668A:pjb:RAVEN:479-2013:6/22/82

REVISED:HED/TOX:Kociolski/Bailey:DCR-24866:WANG0668A:rfr:RAVEN:479-2018:5/30/82

Subject: 90-Day Dietary Study - Rat (First Test: Range Finding) Final Report. Dietary Range-Finding Study in Rats Comparing 2-Samples of ZR-3210 Technical

Test Compounds: Racemic Fluvalinate Technical
Half-Resolved Fluvalinate Technical

Accession No: 070[illegible]

Testing Facility: International Research and Development Corp.

Study Number: IRDC No. 322-043

Responsible Professionals:

Lucy Brewer - Report Writer
Norman D. Jefferson - Director of Chronic Toxicity
Malcolm Blair - Director of Toxicology Division
Patricia Lang - Study Director

Testing Period: November 12, 1980 - February 16, 1981

Report Submitted to Sponsor: September 15, 1981

Purity of Material with Batch or Lot No:

Racemic Mixture
ZR-3210 Technical (93.8%)
Run 7, Analytical No. 0979-069

Half-Resolved Mixture
ZR-3210 Half-Resolved Fluvalinate
(445-95) No. 1080-091. Purity is 89.9%

ZR-3210 Half-Resolved Technical Fluvalinate
Analytical NO. 1280C70, Lab No. 468-27
Purity is 88.2%

Homogeneity, Stability and Concentration in Feed:

A reading of the submission by this reviewer has established that there is a homogeneous distribution of the compound in the diet, the compound is stable in the diet under the conditions of the test and that concentrations in the diet are within acceptable target concentrations.

Reviewers Position and Comments on the Submitted Study:

A reading and visual review of the 90-day range finding study has been conducted by Albin B. Kocialski of the Toxicology Branch.

This reviewer has concluded that the written reviews by Albin B. Kocialski of the non-range finding studies referenced here as,

- Racemic Technical Mixture, 13 Week Dietary Feeding Study in the Rat, IRDC Study No. 322-032, June 19, 1980. Accession No. 070097. Classification: Core Minimum.

- Half-Resolved Technical Mixture. 90-Day Dietary Feeding Study in the Rat. Full FIFRA Protocol, IRDC Study No. 322-047. Accession No. 070662. Classification: Core-Guideline.

supersede and are superior in terms of the quantity and quality of the information provided to the submitted range finding study.

Additionally, any information provided in the range-finding study is already reflected in the two referenced studies on which detailed written reviews are available.

Therefore, as it has been determined by this reviewer that a detailed written review of this 90-day range finding study would add or reveal no new toxicological information not already reflected in the above referenced studies the range-finding study will not be reported by this reviewer (Albin B. Kocialski) as a formal written Toxicology Branch review.

#m14

Subject: 90-Day Dietary Study - Rat (Second Test: Full FIFRA Protocol). Final Report. Final Review.

002256

Test Compound: ZR-3210 Technical (Half Resolved)
Mavrik (R) Technical
Fluvalinate

Accession No: 070662

Testing Facility: International Research and Development Corp.
Mattawan, Michigan

Project No: 322-047

Responsible Professionals:

Barry W. Benson - Director of Quality Assurance
Ward R. Richter - Director of Pathology Division
Joanne R. Kopplin - Senior Staff Pathologist
Norman D. Jefferson - Director of Chronic Toxicity
Malcolm Blair - Director of Toxicology Division
Patricia L. Lang - Study Director
Peter James Dyck, M.D. - Director, Mayo Clinic, Peripheral Nerve Laboratory
(See addendum)

Testing Period: December 15, 1980 - March 16-18, 1981

Report Submitted to Sponsor: September 24, 1981

Purity of Test Material with Batch or Lot No:

455-95. Anal. No. 1080-91. 89.9%
468-27. Anal. No. 1280070. 88.2%
Run 23. Anal. No. 0281028. 93.1%

Homogeneity, Stability, Concentration:

Data was presented which showed compound stability, mixing homogeneity and analytical concentrations within acceptable ranges of the nominal concentrations.

Materials and Methods: Test Article: The test article was offered in the diet on a mg/kg/day basis. The concentrations were recalculated and the diets mixed weekly. Appropriate amounts of half-resolved ZR-3210 were weighed and mixed with ASC grade acetone and Certified Rodent Chow #5002(R). During the first 8 weeks, current body weight and food consumption data as well as data from a range finding study were subjectively used to determine the appropriate dietary concentration of the test material. After study week 8, the following formula was utilized:

mg. test article/kg diet =

$$\text{dosage level (mg/kg/day)} \times \frac{100}{\%\text{ purity of test material}} \times \frac{\text{group mean body wt. (g)} + \text{change in group mean body weight from previous week (g)}}{\text{average food consumption during previous two study weeks (g/day)}}$$

Two hundred fifteen (215) male and 216 female 21-day old Charles River COBS CD(R) rats were shipped from the Charles River Breeding Laboratories, Portage, Michigan, and received at IRDC on November 24, 1980. Animals were held in quarantine for 20 days to assess the general health, and to establish baseline data for body weights and food consumption to be used at study initiation.

A general health screen was performed to determine the status of the animals. Ten male and 10 female animals were randomly selected from the general population for testing. A portion of blood was sent to Microbiological Associates (Bethesda, Maryland) and screened for the following viruses:

Pneumonia virus of mice
Reovirus type 3
Encephalomyelitis
Kilham rat virus
Toolen H-1
Sendai
Mouse adenovirus
Mouse hepatitis
Lymphocytic choriomeningitis
Rat coronavirus
SDA

Blood was also drawn from an additional sample group of 10 rats/sex for the evaluation of hematological and biochemical parameters. These parameters were the same as those described later in this materials and methods section.

The animals were then sacrificed using carbon dioxide asphyxiation and examined microscopically.

An opthalmoscopic examination was also conducted and all animals with ocular lesions were discarded.

One hundred twenty (120) male (162-204g) and 120 female (132-157g) selected rats were assigned to five test and one solvent control groups using a computer generated randomization procedure. Each group consisted of 20 male and 20 female animals. Dosage levels administered were 0 (solvent control), 0.30, 1.0, 3.0, 30.0 and 50 mg/kg/day; groups 1 thru 6 respectively.

All animals were housed in suspended wire mesh cages and quartered in an environmentally controlled room. Cages were changed every two weeks. The faces of the cage racks were placed 4 feet apart and each rack was rotated according to a predetermined schedule so that racks spent equal amounts of time at all positions in the room. Food and water were freely available at all times. It was noted in the report that water was analyzed on a routine basis for heavy metals, coliform bacteria and pesticides. Records of food and water analysis are available on request.

Metal ear tags with the permanent animal number were placed on the rats the day the study was initiated. These ear tags were verified after the initial tagging, after each cage change. before and after collection of blood and/or urine, and at necropsy.

General Observations. The rats were observed twice daily, seven days a week, for moribundity, mortality and signs of overt toxicity. Signs were recorded on the day observed. Detailed observations were recorded weekly.

Any rat, which was observed as having any sign of a life threatening nature, (i.e. tremors, convulsions, impaired or abnormal movement, bleeding, weight loss of 10% in a 1-4 weeks period, food consumption of less than 100 grams during a week, or a decreasing food consumption trend over a 1-4 week period), was placed on a special surveillance list. All rats on this list were examined once daily by the study supervisor.

Individual body weights and food consumption values were measured and recorded weekly.

Clinical Laboratory Tests: Clinical laboratory parameters were measured on blood and urine taken from 10 rats/sex/group which were randomly selected using a computer generated table of random numbers. Rats were fasted overnight. Blood was obtained via puncture of the orbital sinus plexus at the end of the 12-hour fast. "Fresh" urine was collected between the 12th and 15th hour of fast from animals attended in transfer from their permanent cages to metabolism cages, as well as during their period of residence in these metabolism cages. Water was withheld during this 3-hour period. After the 3-hour "fresh" collection period was over, food and water containers were made available to the animals in the metabolism cages for the remainder of the 24-hour urine collection. The animals were then returned to their permanent cages, receiving the same personal attention noted earlier. The remainder of the 24-hour urine samples were then collected and analyzed.

Hematological determinations included hemoglobin (Hg), hematocrit (HCT) erythrocyte count, total leucocyte count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCMC), differential leucocyte count, and reticulocyte count.

Biochemical determinations included sodium, potassium, chloride, calcium, phosphorous, blood urea nitrogen (BUN), creatinine, alkaline phosphatase, total bilirubin, SGOT, SGPT, LDH, total protein, albumin, globulin (calculated), cholesterol and glucose. Urinalysis included color, appearance, microscopic examination of sediment, specific gravity, volume, pH, protein, glucose, occult blood, nitrites, urobilinogen, ketones, and bilirubin. Fresh urine was used to determine all parameters except volume. The 24-hour urine volume was determined by adding the volume of the "fresh" urine sample to the urine volume collected during the remainder of the 24 hour period.

Pathology: All surviving rats were sacrificed by carbon dioxide asphyxiation and subjected to a complete necropsy under the direct supervision of a veterinary pathologist after 13 weeks of dietary administration of the test article. All animals dying during the course of the study or sacrificed in extremis were similarly sacrificed and necropsied. Animals were examined for external abnormalities, including palpable masses and an examination of orifices. Palpable masses were correlated with subcutaneous findings.

Abdominal and thoracic cavities were examined and the organs removed, examined, weighed where appropriate, and placed in a fixative. The brain and pituitary were also examined.

It was also reported that, at the termination of the study, the order of sacrifice, necropsy and the removal and weighing of organs was alternated (one animal from each dose group, then repeating) in order to insure technician consistency in observations, tissue trimming and the weighing of organs. Liver, kidneys, heart, testes, brain (with stem) and ovaries were trimmed free of fat and connective tissue and weighed. Weights were also obtained for any other defined organ having as significant difference in size at necropsy.

The following tissues from all animals in the control and high dose level groups were collected and fixed in neutral buffered formalin. Hematoxylin and eosin stained paraffin sections were prepared from them and microscopically examined by an IRDC staff pathologist.

Adrenal (both
Brain (3 levels - fore, mid, and hind brain)
Eyes and contiguous Harderian glands (both)[1/]
Gonads
Ovaries
Testes with epididymis[1/]
Heart (with coronary vessels)
Esophagus
Stomach
Small intestine (jejunum)
Large intestine (colon)
Kidneys (both)
Urinary bladder[3/]
Prostate/corpus and cervix uteri
Liver (2 lobes)
Lung and mainstream bronchi (2 coronal sections including all lobes)[2]
Lymph nodes (mediastinal, mesenteric, and regional)
Mammary gland
Mandibular salivary gland
Sciatic nerve
Pancreas
Pituitary
Skin
Spinal cord (cervical, thoracic, and lumbar)
Spleen
Thymus (where present)
Trachea
Thyroid/parathyroid
Sternum (bone marrow)
Skeletal muscle
Any other tissue (s) with lesions (to include a border of apparently normal tissue)

In addition, the sural (calf) nerve from the randomly selected animals per sex group was fixed in situ in 2.3% glutaraldehyde, 2% paraformaldehyde, dissected out, and stored in 0.1 m cacodylate buffer. The evaluation of the sural nerve was conducted by and the report prepared by Peter James Dyck, M.D., who is the Director of the Peripheral Nerve Laboratory of the Mayo Clinic in Rochester, Minnesota (see addendum).

From all animals in the intermediate dosage level groups, the following tissues were prepared and microscopically examined as described above.

1/ Eyes and testes were fixed in Bouin's fixative.

2/ Lungs were inflated with formalin via the trachea.

3/ Urinary bladders were inflated with formalin and left unopened for examination following fixation.

Kidneys	Brain
Liver	Skeletal muscle
Heart	Gross lesions
Spinal cord	Any target organs noted in the high dosage level group
Sciatic nerve	

Inadvertent Errors: During the necropsy tissues from animal number 68393 (Group 1, female) and animal number 68533 (Group 5, male) were placed in the same container. Some tissues were adequately identified. Only one lesion was found, a trace of focal chronic nephritis, and it appeared in a clearly identified tissue (animal number 68533). All other examined tissues were within normal limits. It was therefore concluded that this inadvertent error had no scientific impact on this study (note: this reviewer sees no reason to disagree).

The protocol specified that the right and left eyes be identified. The eyes were inadvertently placed in the same block and their specific identity could not be determined. Lesiors in the eye or associated lacrimal gland occurred sporadically among the control as well as the treated animals. This inadvertent error had no bearing upon the scientific validity of the study.

Some errors in the transcription of numerical values were observed within the report. However, this reviewer has determined that these errors were minor and do not affect the integrity or conclusions of the report.

Opthalmoscopic Examinations: Opthalmic examination (binocular indirect opthalmoscope with a positive 20 diopter focusing and magnifying lens) of the rats was performed during the pre-test period and at week 12.

Statistical Analysis: All statistical analyses compared the treatment groups with the control group, by sex.

Body weights (weeks 1-13), food consumption (weeks 1-13 grams/rat/week), hematological, biochemical and select urinalysis parameters (weeks 6 and 13) and absolute and relative (to both body and brain weights) organ weights (terminal sacrifice) were compared by analysis of variance (one-way classification), Bartlett's test for homogeneity of variances and the appropriate t-test (for equal or unequal variances) as described by Steel and Torrie using Dunnett's multiple comparison tables to judge significance of differences.

Pre-Test General Health Screen

Hematological, biochemical, viral, ophthalmological and pathological evaluations conducted during the pretest period indicated that the population of animals obtained for use in this study was suitable for testing. During the pretest viral screen, about half of the animals showed low titres for KRV virus, but this virus has not been shown to influence any of the parameters evaluated in this study. At study termination, titres of Sendai, RCV and SDA were found in most animals examined. These are common viruses found in laboratory rats and their presence would not be expected to influence the findings in this study.

RESULTS

General Observations: Salivation in males (8/20) at the high dose during study weeks 1-13 was the predominant sign observed. The number of males showing this sign greatly diminished with time (3/19). Salivation in females (3/20) was also observed in the high dose group during weeks of 1-3 but to a much lesser extent than males. The number of females showing this sign was also diminished with time. Salivation was not (for all practical purposes) seen in either sex at lower dose levels. An abnormal gait was observed in both sexes in the high dose group only. Seven of 20 males and 7/20 females manifested an abnormal gait during weeks 1-3. The number of animals of either sex showing an abnormal gait greatly diminished with time (1/19). An abnormal gait was not noted at lower dose levels. Deaths for males and females did not exceed 10% and were generally confined to the high dose group. Only one death occurred at the lower dose levels and that was a female at 1.0 mg/kg.

Skin lesions were observed in both sexes. The salient summary data are reproduced below:

Skin Lesions

Males

Study Weeks Dose (mg/kg/day)	1-3	4-9	10-13
3.0	4/20	0/20	0/20
30.0	14/20	16/20	12/20
50.0	3/20	10/19	7/18

Females

Study Weeks Dose (mg/kg/day)	1-3	4-9	10-13
30.0	12/20	15/20	10/20
50.0	10/20	10/19	10/19

Body Weights: Males: Mean body weights were not statistically significantly different between controls and the three low dose groups. Body weights were statistically significantly lower ($p<0.01$) at the 30 and 50 mg/kg/day dose levels for all time periods. Females: Mean body weights were not statistically significantly different between controls and the three low dose groups. Body weights at 30 mg/kg/day were statistically significantly different

($p<0.05$) at only four time periods--2, 4, 9, and 12 weeks. However, body weights were substantially depressed at all other time intervals. Body weights were statistically significantly lower ($p<0.01$) at all time periods in the high dose group, with the exception of the reading at the second week which was comparable to controls.

The percent differences in mean body weight from controls for the treated groups at week 13 were as follows:

Sex / Dose (mg/kg/day)	Males	Females
0	-	-
0.3	-1.2	-1.4
1.0	+0.6	+0.3
3.0	+1.2	-4.9
30.0	-15.8	-6.3
50.0	-25.3	-11.9

Mean Food Consumption: Males: Mean food consumption was comparable between controls and the three low dose groups. Food consumption was generally depressed and statistically significantly lower ($p<0.01$) at weeks 1, 2, 4, 8, 9, and 10 for those animals receiving 30 mg/kg/day. Food consumption was statistically significantly lower ($p<0.01$) at all time periods in the high dose group. Females: All groups with the exception of the two high dose groups were comparable to control values. Food consumpton in the 30 mg/kg/day group showed values lower than controls which were statistically lower ($p<0.05$) at two time periods - the 1-week reading and the 9-week reading. Values for the high dose group were generally depressed for all time periods with statistical significance being noted at 1, 6, 8, 9, and 10 weeks.

The average food (noted here as a percent change) and compound consumption values (absolute values mg/kg/day) are noted as follows:

	Food Consumption		Compound Consumption	
Dose	M(%)	F(%)	M	F
0	-	-	-	-
0.3	-1.6	+0.5	0.3	0.3
1.0	+2.4	+2.2	1.0	1.0
3.0	+1.2	-2.7	3.0	2.9
30.0	-10.8	-5.4	30.0	30.0
50.0	-22.8	-12.9	50.0	50.0

Hematology: Only those parameters showing a statistically significant difference, as well as those parameters judged by this reviewer to be immediately relevant to those tabulated as statistically significant, are noted below.

Males at 6 Weeks

Dose (mg/kg/day)	0.3	1.0	3.0	30	50
Parameter					
Neutrophils[1/]			I	I*	I
Lymphocytes				D**	D
Erythrocytes				D	D*
HCT				D	D*
Hg				D	D

Males at 13 Weeks

Dose (mg/kg/day)	0.3	1.0	3.0	30	50
Parameter					
Leucocytes	D*			I	I
Neutrophils[1/]				I*	I
Lymphocytes				D*	D
Hemoglobin (Hg)				D*	D*
HCT				D*	D*
Platelet				I*	
MCV					I**
Erythrocytes				D	D**
MCH					I*
Reticulocytes				I	

* = $p<0.05$ D = Decrease
** = $p<0.01$ I = Increase

[1/] Segmented

Females at 6 Weeks

Dose (mg/kg/day)	0.3	1.0	3.0	30	50
Parameter					
Neutrophils[1/]				I	I*
Lymphocytes				D	D*
Erythrocytes		I*		D**	D**
Hg				D**	D**
HCT				D**	D**
Platelets				I*	I*

Females at 13 Weeks

Dose (mg/kg/day)	0.3	1.0	3.0	30	50
Parameter					
Neutrophils[1/]				I*	I*
Lymphocytes				D*	
Leucocytes	D*				I*
Erythrocytes			D	D*	D**
Hg				D*	D**
HCT				D**	D**
Platelets				I	I*

Clinical Chemistry: Only those parameters showing a statistically significant difference, as well as those parameters judged by this reviewer to be immediately relevant to those tabluated as statistically significant, are noted below.

Males at 6 Weeks

Dose (mg/kg/day)	0.3	1.0	3.0	30	50
Parameter					
Alkaline Phosphatase		I*			
Calcium				D	D
Total Bilirubin				D*	
Albumin				D*	D*
Total Protein					D**
BUN				I	I

Males at 13 Weeks

Dose (mg/kg/day)	0.3	1.0	3.0	30	50
Parameter					
Calcium				D**	D**
Total Bilirubin				D*	D**
Albumin				D*	
Total Protein					D**
BUN				I	I
Globulin					D*
Glucose				D**	D**
SGPT				I*	

* = $p<0.05$ D = Decrease
** = $p<0.01$ I = Increase

Females at 6 Weeks

Dose (mg/kg/day)	0.3	1.0	3.0	30	50
Parameter					
Albumin				D**	D**
Total Bilirubin				D*	D**
BUN				I**	I**

Females at 13 Weeks

Dose (mg/kg/day)	0.3	1.0	3.0	30	50
Parameter					
Albumin				D*	D**
Total Bilirubin				D**	D**
BUN				I*	I
SGOT					I*
Creatinine	I*				I*

Organ Weight to Body Weight Ratios:

Males

Dose (mg/kg/day)	Liver	Kidney	Testes	Heart	Brain
0.3				I*	
1.0		I*			
3.0					
30	I**	I**	I**	I*	I**
50	I**	I**	I**		I**

Females

Dose (mg/kg/day)	Liver	Kidney	Ovaries	Heart	Brain
0.3		I*			
1.0					
3.0					
30	I**	I**			
50	I**	I**			I*

* = $p<0.05$
** = $p<0.01$

I = Increase

Organ Weight to Brain Weight Ratios:

Males

Dose (mg/kg/day)	Liver	Kidney	Testes	Heart
0.3				
1.0				
3.0				
30		I*		
50		D*		D**

Females

Dose (mg/kg/day)	Liver	Kidney	Ovaries	Heart
0.3				
1.0				
3.0				
30	I	I**		
50	I*		D**	

* = $p<0.05$
** = $p<0.01$

I = Increase
D = Decrease

Urine Volume: Males at 6 weeks: All values appear generally comparable up to 3.0 mg/kg/day. A downward trend begins at 30 and is statistically significant at 50 mg/kg/day.

Males at 13 weeks: No statistically significant differences were noted at any dose level. A decreased volume was noted at 50 mg/kg/day.

Females at 6 and 13 weeks: No statistically significant decrease at any dose level. All values appear to be comparable between groups.

Urine Acidity Conducted on Fresh Morning Urine (pH): Males at 6 weeks: All values comparable to controls for all dose levels.

Males at 13 weeks: All values comparable to 30 mg/kg/day. No data available at 50 mg/kg/day.

Females at 6 weeks: All values comparable at week 6.

Females at 13 weeks: All values comparable to 3.0 mg/kg/day. No data available at 30 and 50 mg/kg/day.

Other Urine Parameters: All other urine parameters appeared to be within normal range.

Eye Examination: Lid erosion was noted for two 50 mg/kg/day females at the end of the study.

Viral Screen: Titres of Sendai, RCV, and SDA viruses were found in most animals examined. These are common viruses found in the laboratory.

Pathology (Gross): The gross pathology for those animals dying intercurrently or sacrificed prematurely can be considered as not remarkable. The primary observations reported at terminal sacrifice for both sexes were skin lesions and regionally enlarged lymph nodes.

Males

Regionally Enlarged Lymph Nodes

Dose (mg/kg/day)	Responding (Animals)
30	8/20
50	3/18

Skin Lesions

30	13/20	(7/13 scabbed/ulcerated skin)
50	11/18	(6/11 scabbed/ulcerated skin)

Females

Regionally Enlarged Lymph Nodes

Dose (mg/kg/day)	Responding (Animals)
30	8/20
50	6/19

Skin Lesions

30	14/20	(9/14 scabbed/ulcerated skin)
50	16/19	(11/16 scabbed/ulcerated skin)

Note: Control animals were free of skin lesions and regionally enlarged lymph nodes.

Pathology (Microscopic): The pertinent results of the histopathological examinations at terminal sacrifice are noted as follows:

Regionally Enlarged Lymph Nodes - Lymphadenitis

Males

Dose (mg/kg/day)	Observations - No. Inflamed/No. Examined)
30	11/11
50	4/5

Females

Dose (mg/kg/day)	Observations
30	9/9
50	5/6

Skin

Males

	30 mg/kg/day	50 mg/kg/day
Dermititis	11/12	7/18

Females

Dermititis	10/10	10/19

Note: Control animals were free of skin lesions and enlarged regional lymph nodes (lymphadenitis).

The only other apparent noteworthy findings were observed in the high-dose females where 4/19 females manifested erythroid hyperplasia of the sternum and 1/19 females in the high dose showed an extramedullary hematopoiesis of the spleen.

It is also noted here that there were no adverse pathological findings reported for the livers of either sex. The pathological examination, therefore, appears to negate the increases reported for the organ to body/brain weight ratios reported for females at 30 and 50 mg/kg.

Discussion: The signs of abnormal gait and salivation appear to be inter-related events associated with compound administration and are interpreted by this reviewer as an expression of nausea (or gastro-intestinal irritation) with a secondary effect of an abnormal gait.

It is unclear to this reviewer as to whether or not the body weight decreases were secondary effects resulting from a decrease in food consumption (i.e., an increase in the log-dose of active ingredient results in a log-dose response decrease in body weight due to a log-dose response decreased palatability of the diet) or that body weight decreases were a direct result of compound

ingestion (i.e., log-dose of compound administered and ingested results in a log-dose response decrease in body weight). It has been shown that the ingestion of active ingredient calculated on a weekly basis had a 13 week mean value of 30 mg/kg/day (37-26 gram range) and 50 mg/kg/day (56-44 gram range). It is the opinion of this reviewer that a total or partial resolution of this issue of body weight decreases may well come from the two year rat study (method of oral administration being gavage) now in progress. As no currently recognizable systemic effects (other than those appearing secondary to skin lesions) were observed at any dose, one could argue that the decreases in body weights are the result of a decreased palatability of the diet. It is known from another study (dog) that the palatability of food decreases when the compound is added to the diet. The data do not appear to suggest that skin lesions are related to decreased food intake and decreased body weight as no log-dose response was observed between skin lesions and/or body weight and food intake (see also below).

The argument (originally presented by the contractor and with which this reviewer tends to agree) that the lesions were the result of a response to a topical irritation rather than a systemically induced effect is as follows: (i) The incidence of animals with skin lesions was much higher in the 30 mg/kg/day dose group than in the 50 mg/kg/day dose group during the first few weeks of the study, even though the animals in the 50 mg/kg/day dose group ingested approximately twice as much test article on an mg/kg/day basis during this time (based on actual test article consumption calculations, see attached), and (ii) the animals in the 50 mg/kg/day dose group consumed less total diet on a gram/rat/day basis than rats in the 30 mg/kg/day dose group during the first few weeks of the study, which may have reduced their opportunity for dermal contact with the test article. Deaths did occur primarily in the high-dose group and most likely were the result of compound administration.

Clinical chemistry, hematological and pathology data are mutually supportive and are indicative of an anemia at dose levels of 30 and 50 mg/kg/day. The contractor has indicated that the skin lesions at these dose levels were of a sufficient size and severity (and the submitted data do seem to reflect this) to affect the hematological and clinical chemistry parameters. This reviewer does not disagree with this position. However, we take this opportunity to state that the Agency has requested that the registrant in their interim report(s) of the chronic feeding study (conducted in rats with compound administered by oral gavage) separate findings of lesions (if any) from those animals not showing lesions, as well as to separate the results of hematological and clinical chemistry findings from those animals showing and not showing lesions. We have asked that this be done to assure ourselves that the lesions are not masking the effects of a true anemia.

Hematological, clinical chemistry and pathlogical data also indicated the presence of skin lesions with attendant tissue destruction. It was reported that the lesions in the skin were characterized by focal ulceration and inflammation involving the dermis, subcutis and extending to the muscularis. The inflammatory cells consisted primarily of lymphocytes, plasma cells and fibroblasts with polymorphonuclear leukocytes. Proteinaceous exudate and thrombi occasionally adhered to the areas of ulceration. Less severe lesions were characterized by hyperkeratosis, perakeratosis or inflammatory cell infiltration into the dermis and subcutis beneath an intact epithelial lymph node. Changes in the enlarged lymph nodes were consistent with those of a

reactive lymphoreticular organ; lymphoid hyperplasia, sinus histiocytosis, fibrosis and erythrophagocytosis.

Organ to body weight ratios were statistically significantly increased in both sexes at 30 and 50 mg/kg/day. These differences generally disappeared when organ to brain weight ratios were calculated. It was therefore concluded that the increase in body weight/organ weight ratios was a result of decreased body weight. Additionally, there were no adverse pathological findings reported for the livers of either sex. The pathological examination therefore appears to negate the liver weight increases reported for females (organ weight/brain weight ratio) at 30 and 50 mg/kg.

NOEL: Lesions of the skin: 1.0 mg/kg/day.

NOEL: Systemic: 3.0 mg/kg/day

Classification: Core-Guideline

ADDENDUM

Subject: Neuropathological Evaluation of Rats at Termination of the 90-Day Study. (Second Test: Full FIFRA Protocol). Examination of the Sural (Calf) Nerve, a Peripheral Nerve.

Test Compound: Mavrik(R) Technical Fluvalinate
ZR-3210 Technical (Half-Resolved)

Accession No.: 070663 (See also Acc. No. 070662 which contains the full review of the study.)

Study Number: IRDC 322-047

Responsible Professional: Peter James Dyck, M.D., Director, Mayo Clinic, Peripheral Nerve Laboratory, Rochester, Minnesota.

Summary and Conclusion: During the terminal sacrifice, nerves from 10 randomly selected male and female rats from the vehicle control group, the 30 mg/kg/day and the 50 mg/kg/day groups were harvested by personnel from the Mayo Clinic Peripheral Nerve Laboratory. Three nerves were obtained from each of 20 rats per dose group. Although the nerves from rats of the 30 mg/kg group were harvested and histologically processed, they were not evaluated further. The design of the study was that they be evaluated only if a pathological abnormality was observed in the 50 mg/kg group.

At the time of harvesting of nerves, the rats were anesthetized with pentobarbital. The sural nerve was taken following fixation in situ both at mid-thigh level and at the ankle level. The peroneal (i.e., pertaining to the fibula or to the outer side of the leg) nerve just above the knee was also taken. Nerves were fixed in situ using glutaraldehyde, in buffered 0.025M cacodylate buffer for a period of 10 minutes and were then immersed after removal in 2.5% glutaraldehyde in the same buffer. Following a 24-hour period of fixation they were washed, osmicated, and embedded for transverse sections into epoxy. Transverse 0.75 um sections were cut and stained with phenylenediamine and others with methylene blue. All histology procedures were performed by personnel from the Mayo Clinic Peripheral Nerve Laboratory.

The sections were inspected at a low (x 125) and at a high (x 300) magnification on a blind basis (i.e. examiners did not know which tissues came from control or treated animals). For each section, notes were kept regarding density of myelinated fibers, size distribution, the presence of segmented demyelination or remyelination, the presence of axonal degeneration, the occurrence of axonal organelles and other pathological findings.

An abnormality of density, size distribution, and axonal organelles was not observed for any of the sections of the nerves. It was reported that an occasional fiber may have been undergoing axonal degeneration, but this was very difficult to discriminate from a normal paranodal region. Three types of abnormalities were observed and recorded under "other pathological findings." Most commonly seen was irregularity of the myelin profile. Such fibers appear crenated (i.e. scalloped or notched). Another common abnormality was splitting of the myelin. A third abnormality was a change described as "crush artifact" and was attributed to handling of the tissue before it was adequately fixed.

Following the microscopic evaluation of all the tissue sections, the identity of each of the nerves was provided (i.e. each tissue was identified as to whether it came from the control group or the treated group) and the results of the two groups were compared. Comparison of the results made clear that the changes which were recorded under "other pathological findings" occurred as frequently in control as in the treated group nerves.

The pathological review of the transverse sections of sural and peroneal nerves did not show a greater abnormality in the nerves of the fluvalinate fed (treated) rats than from control rats.

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REVISED:KOCIALSKI/BAILEY:DCR-07904:WANG-1009B:pjb:RAVEN:479-2013:5/18/82 002256

RESULTS

180-Day Dog Study

The results and discussion of this review cover the experimental period between days 90 and 180 with the exception of skin lesions (clinical observations) which cover the period 1 thru 180 days. This report is (or should be) found appended to the results and discussion which cover the days 1 thru 90 of the 180-day dog study.

REFERENCES

Subject: Six-Month Subchronic Dog Study. Final Report.

Test Compound: ZR-3210 Technical (Racemic Mixture), Fluvalinate, Mavrik®

Accession Nos: 070097- 98; 99

Testing Facility: Elars Bioresearch Laboratories, Inc., Fort Collins, Colorado 80524

Project No: 1503

Testing Period: December 21, 1979 - April 13, 1980

Report Submitted to Sponsor: June 1980

Purity of Test Material: 93.8% (first shipment); 93.3% (second shipment)

Batch or Lot No: Analysis No. 0979-069 Run 7 (first shipment); Analysis No. 0280092 (second shipment)

Stability: Stable for at least 12 months when stored in sealed glass containers and exposed to artificial light at 25 and 42°C. Stable in preparation and under the conditions of the study.

Clinical Observations: Emesis: The most frequently detected dose-related sign was emesis which occurred frequently in all dogs and almost daily in some dogs receiving 50 mg/kg/day. Accomodation to the test material in terms of a decreased frequency of emesis was not observed. Emesis occurred infrequently in the control group and generally to the same extent in those animals receiving 2 and 5 mg/kg/day. The frequency and quantity of emesis in animals receiving 15 mg/kg/day generally ranged from 2-15 times greater than that seen at the lower dose levels. The frequency and quantity of emesis in dogs receiving 50 mg/kg/day was 5-13 times greater than that seen at 15 mg/kg/day.

Diarrhea: The frequency of diarrhea between the control group and the two lower dose levels was generally comparable. The frequency of diarrhea at a dose level of 15 mg/kg/day was generally 9-20 times greater at this dose level than at the lower dose levels and 2-12 times greater at 50 mg/kg/day than that seen at 15 mg/kg/day.

Dehydration: In the Toxicology Branch review of the 90-day interim kill report to the 180 day dog study, it was noted that dehydration occurred in several dogs receiving the high dose and appeared consistently in 4 females and 1 male. Three of these dogs (all females) were sacrificed at the 90-day mark. The male (MH-103) which had received lactated Ringers Solution as an emergency treatment for one week remained somewhat dehydrated but stable and responsive through out the remainder of the study. The female (WZ-89) did not receive lactated Ringers Solution and did remain somewhat dehydrated (0-5%), but stable and responsive through the 180 day period.

During week 18 of the study, one female (UB-89) from Group IV (15 mg/kg) became severely depressed and dehydrated. The animal's condition deteriorated steadily, with rapid weight loss, anorexia, and the appearance of multiple abscesses. The animal was euthanatized on day 159 of the study.

Skin Lesions: Localized skin lesions persisting for several weeks were reported in all groups receiving the test article (see attached). Although a log-dose response was not evident, a compound related effect appeared to be present. A tabular representation of data for those animals showing at least one lesion at any time period from 1 to 180 days is reproduced below.

Number of Lesions Observed at Any Time Period (180 Days)

Group	Male	Female	Total
I	0	0	0
II	0	1	1
III	2	5	7*
IV	2	3	5
V	4	4	8

* One male had OTITIS prior to testing and was erroneously counted as having a lesion at 90 days. This number is one less than reported at 90 days.

One male and one female each in group IV and V which did not manifest lesions during the 1-90 day period did manifest lesions after 90 days.

Lesions were generally described as an open sore, irritation, or inflammation. Open sores were generally in the neck area or the right hind paw. Irritations were associated with the prepuce and vulva. Inflammation and/or irritation were terms used to describe findings in or on the ears.

Examination of the table shows that the number of lesions appeared to be nearly equally distributed between sexes and that no lesions were recorded for controls.

One female (UB-89) of Group IV had an open sore of the neck 60 days post dosing which lasted for 8 weeks. This animal was treated with nitrofurazone powder. Approximately 75 days later this same animal manifested abscesses of the face, neck, and right front paw. Bacterial culture results were negative for the neck but positive for the face (unidentified species of Staphylococcus). No culture results were taken of the paw. This animal (UB-89, see also dehydration paragraph) was sacrificed at 159 days due to a severe deterioration of its general health.

It was reported that dogs with lesions appeared to suffer from pruritus; they licked, chewed or scratched the affected areas repeatedly. The pruritus appeared to be more severe shortly after dosing, since dogs which were quiesccent in the morning chewed and scratched themselves two to three hours after they were dosed.

Numerous treatments were attempted. The affected areas were wrapped or bandaged, Elizabethan collars were placed on the dogs and finally, if necessary, various topical medications were applied. The application of medication (topical antibiotic) was reserved only for the most refractory cases. Staphyloccocus aureus was identified in 3 dogs and beta-hemolytic streptococcus in 2 dogs. Staphylococcus, unidentified as to species, was also diagnosed in a sixth dog.

Body Weights: Mean body weights for males in the high dose group revealed statistically significant ($p < 0.05$) decreases from week 19 to 26 inclusive when compared to the control group. Values for females were not statistically significantly lower at any time period. The combined male and female body weights were not statistically significantly lower than combined control weights at any time period.

Graphical representation of male body weight data shows parallel increases in body weight and non-significant departures from controls for Groups I thru IV thru termination. Group V, the high dose group, departs from all other groups at about 6 weeks and remains flat for the remainder of the study.

Graphical representation of female body weight data shows parallel and non-significant divergence from controls for Groups III and IV while Group II shows parallelism and is substantially above the control group. Body weights for females of Group V are essentially flat for weeks 1-4. Body weights for weeks 4-26 are parallel but substantially below controls.

The total weight gain for males in the 50 mg/kg/day dose group was statistically significantly lower at the end of 26 weeks when compared to all other groups.

The total body weight gain for females in the 50 mg/kg/day dose group was substantially lower than all other groups but the decrease was not statistically significant.

The combined values for sexes were substantially lower for the 50 mg/kg/day group, but not statistically significant. All other group values were comparable to control values.

Food Consumption: Food consumption was not statistically significantly different from control values for either sex when considered separately or in combination.

Opthalmic Examinations: Terminal opthalmic examinations were performed on all surviving dogs prior to necropsy. The examinations were reportedly carried out on a blind basis and subsequently compared to the 90 day examination. No significant changes from the 90 day examination were observed. No substantial changes were observed in the results from the Schirmer tear test.

Neurologic Examination: Observations reported for animals at 180 days (note here that only three groups were examined for neurologic abnormalities - they were the vehicle control group, the 5.0 mg/kg/day group and the 50.0 mg/kg/day group) revealed the following; 12/12 control animals were reported as normal; 12/12 dogs in the group receiving 5.0 mg/kg/day were reported as normal; and 11/12 dogs in the high dose group showed no abnormalities. Only one animal, a female, which showed hyporeflexia of the right and left rear patella at 90 days continued to show the same sign at 180 days.

Clinical Pathology: Hematology: The Red Blood Cell Count (RBC) for males and females of the high dose group at 180 days, although not statistically significant, was much lower than control values. However, the combined values for males and females for the same dose and time period were statistically significantly ($p < 0.05$) lower when compared to the combined values for the control group. The hematocrit was statistically significantly lower in the high dose group for the combined male and female values at the 180 day reading. However, values for males, females and their combined values were depressed when compared to control values at all time periods (i.e., 120, 150 and 180 days) for the high dose (50 mg/kg/day) group. Hemoglobin values for male and female animals showed successive decreases at 180 days with increased dose. None of the values were however statistically significant. The combined values for males and females showed successive decreases in hemoglobin (Hg) values with an increased dose at 180 days. The dose of 50 mg/kg/day produced a statistically significant decrease. Platelet count was not statistically significantly different at any dose level at any time period. Activated Partial Thromboplastin Time (APTT) and Protime (prothrombin time) values were comparable to control values.

No statistically significant changes were noted for values of mean corpuscular hemoglobin. The mean corpuscular hemoglobin concentration was statistically significantly lower than Groups I, II and III for males in the high dose group at 180 days. No other biologically meaningful changes were observed at any time period. White blood cell count (WBC) and WBC differential count did not appear to show any biologically meaningful differences in the context of compound administration even though some values were statistically significant. These changes occurred randomly. Methemoglobin values taken on day 150 only were not statistically significantly different at any dose level for males or females when considered either separately or together. Total protein was statistically significantly decreased at various time intervals and doses. However, no log-dose response was evident and no biologically

meaningful decreases with time were observed. The statistically significant decreases appeared to occur in a random manner. Albumin values for males were statistically significantly decreased in the high dose group at 180 days. Values for females were statistically significantly decreased at 120 and 180 days in the high dose group. Values for females were depressed in the high dose group at 150 days when compared to controls. The combined values for males and females, when compared to controls, were statistically significantly lower than controls at all three time periods (i.e., days 120, 150, and 180) in the high dose group. Additionally, combined values in the high dose group were statistically significantly lower when compared to two other dose groups, beside control group, at the 120 and 180 day reading. Globulin values were statistically significantly increased only for the combined values for sexes at 120 days in the high dose group when compared to controls.

The albumin/globulin ratio (A/G ratio) was statistically significantly decreased only for females and the combined values for males and females at 120 days in the high dose group. Values for glucose were not statistically significantly different from control values at any dose level or time period. Values for Blood Urea Nitrogen (BUN) were not statistically significantly different from control values at any dose level or time period. Total bilirubin was comparable for all groups at all time intervals. Alkaline phosphatase values were comparable for all groups at all time intervals. Calcium values at 120 days were statistically significantly decreased in the high (50.0 mg/kg/day) dose group for males and the combined values for males and females. Calcium values were also statistically significantly lower at 180 days for the combined values of the sexes at the high dose. It is also pointed out here that inspection of the data reported for 180 days revealed lower calcium values in the high dose group as apposed to all other groups for both males and females. Values for 150 days appeared comparable.

Values for Na and K were not statistically different between groups at any time period. SGOT and SGPT values for males and females were not statistically significantly different between groups at any time period. Cholesterol values were comparable for treated groups when compared to controls at all time periods. LDH values were comparable between treated groups and control groups. RBC cholinesterase corrected values were not statistically significantly decreased

at any time period for any test group [Note: corrected values were reported for 60, 90 and 180 days. Only absolute values were originally reported for 60 and 90 days and showed values to be statistically significantly lower for males, females and their combined values at 90 days. This reviewer argued that the decreased RBC cholinesterase values at 90 days were probably the result of a decreased hematocrit. The absolute RBC cholinessterase values corrected for a decreased hematocrit reveal no values statistically significantly different from the control group. Corrected values were calculated by dividing the mean control hematocrit by the individual hematocrit and multiplying by the absolute RBC cholinesterase value for each dog]. Serum and brain cholinesterase values were comparable at all doses and time intervals. Values for specific gravity (urine) and ph (urine) were presented for combined sexes and were not statistically significantly different from the control group values at 120, 150 and 180 days. No statistically significant differences were noted among groups for the other parameters listed at 120, 150 and 180 days for urinalysis.

The absolute terminal body weights for male dogs were statistically significantly lower only for the high dose group when compared to the control values.

Absolute organ weights were not significantly different between treated animals and controls. Organ to body weight ratios were statistically significantly greater for liver, kidney and adrenal only for males in the high dose group when compared to any other group. Organ to brain weight ratios were statistically significantly greater for males in the high dose group only for liver. Brain weight ratios for kidneys, liver and adrenals were not statistically significantly increased when compared to controls at dose levels of 15 and 50 mg/kg/day for females.

Pathology: The salient pathological findings are noted below with their respective doses for dogs continued on the experiment after 90 days.

> Dose: 5.0 mg/kg/day. Male dog DK 99. Skin. Right hind paw lesion and left leg lesion, hyperkeratosis (moderate severity) inflammation, chronic (moderate severity).
>
> Dose: 15.0 mg/kg/day. Male dog AW 99. Skin paw. Right hind paw, hyperkeratosis (moderate severity), inflammation, chronic (moderate severity). Skin neck, a scabbed sore, inflammation, chronic (moderate severity).

Female dog UB 89. Face, abscess, inflammation, chronic (moderate severity).

Dose: 50.0 mg/kg/day. Male dog MH 95. Paw pad, right hind lesion, hyperkeratosis (severe), inflammation, chronic (moderate severity).

Female dog DH 99. Skin foot, right hind, lesion; inflammation chronic (moderate severity). Skin neck lesion, chronic inflammation of slight severity.

Female dog BX 99. Lesion of left and righ hind paw pads, hyperkeratosis (moderate severity), chronic inflammation (moderate severity).

Male dog CQ 99. Prepuce, raw sore. Epidermal inclusion cyst (slight severity).

The histological appearance was also characterized by the dermal infiltration of neutrophils and lymphoid cells.

SUMMARY

Males: A dose level of 2.0 mg/kg showed no apparent differences for effects from the control group.

A dose evel of 5.0 mg/kg showed no apparent difference for effects from the control group, with the exception of 2 dogs manifesting skin lesions. It is noted here that the number of skin lesions which were observed during the course of the experiment may not have been equal to those tabulated at histological evaluation. This is not unexpected since some lesions healed spontaneously prior to necropsy, some lesions were treated and were healed prior to necropsy and others were observed at necropsy and during histopathological examination.

A dose level of 15.0 mg/kg produced emesis and diarrhea which occurred at a greater frequency than either controls or the lower dose levels. Additionally, two animals manifested skin lesions. No other apparently meaningful effects were observed.

A dose of 50 mg/kg produced emesis and diarrhea which occurred at a greater frequency and quantity than the next lower dose level. One male which had received lactated Ringers solution early during the experiment was somewhat dehydrated but stable and responsive during the course of the experiment. Four males were observed as having skin lesions, one of which persisted to termination (Animal CQ 99, see also attached). Body weights were statistically significantly decreased from week 19 thru week 26. The mean corpuscular hemoglobin concentration was statistically significantly lower than Groups I, II and III at 180 days. Albumin was statistically significantly lower at 180 days. Calcium levels were statistically significantly lower at 120 days. The liver, kidney and adrenals were statistically significantly increased for organ to body weight ratio but only liver was statistically significantly increased for organ to brain weight ratio.

Females: A dose level of 2.0 mg/kg showed no apparent differences for effects from the control group with one exception. One female showed one lesion (open sore of the neck) at approximately 48 days post-dosing. The lesion was observed for 4 weeks but not treated. The animal was observed to scratch this lesion open repeatedly. The animal behavioral response to this lesion was similarly described for other animals at higher doses where a lesion was observed. This lesion apparently healed spontaneously. This lesion was previously reported during the review of the first 90 days of this study.

A dose level of 5.0 mg/kg showed no apparent differences for effects from the control group with one exception which was the manifestation of skin lesions in five animals--four animals manifested lesions during the in-life segment of the experiment and one manifested a lesion during histopathological examination.

A dose level of 15 mg/kg showed emesis and diarrhea occurring at a frequency and quantity greater than that of controls or the lower dose levels.

One female became severely depressed and dehydrated. The general health deteriorated with attendant weight loss, anorexia and the appearance of multiple abscesses (confirmed for staphylococus). The animal was euthanized on day 159 of the study. This was the only animal sacrificed inter-currently.

Three animals of this group manifested skin lesions. One of the animals was the one sacrificed inter-currently.

A dose level of 50 mg/kg manifested an emesis and a diarrhea to a greater extent than seen in controls or the next lower dose level. One female of this dose group remained 0-5% dehydrated for the entire period of the experiment. However, this animal was generally responsive during the course of the experiment and its general health appeared to be good. Four animals manifested skin lesions which were observed during the in-life portion of the experiment. Body weight was generally depressed during the course of the experiment. Total weight gain was also depressed at termination. The albumin level was statistically significantly decreased at 120 and 180 days and depressed at 150 days.

Males and Females Combined Values: Some effects only became apparent when the values for males and females were combined. These events occurred primarily at the high dose. The red blood cell (RBC) count was statistically significantly decreased at 180 days when male and female values were combined at the high dose. The RBC values when viewed separately by sex were generally depressed when compared to control values at 120, 150 and 180 days at the high dose. The hematocrit (HCT) for combined values at 180 days in the high dose group were statistically significantly lower compared to controls. The hematocrit values appeared to be generally depressed for separate sexes at 120, 150 and 180 days and the combined values for sexes at 120 and 150 days. Hemoglobin (Hg) values were shown to decrease with an increased dose for the separate values of sexes as well as the combined values for sexes in the high dose group at 180 days. A statistically significant

decrease was however only observed for the combined values of sexes at 180 days in the high dose group. Albumin values for the combined values of sexes were statistically significantly decreased at the high dose group at 120, 150 and 180 days. Calcium values for the combined values of sexes at 50 mg/kg were statistically significantly decreased at 120 and 180 days.

Discussion: Males and females mainifested emesis and diarrhea comparable to the control animals at doses of 2.0 and 5.0 mg/kg. Increased doses of compound led to a progressive increased emesis and diarrhea which were substantially above control values. It has been shown in previous dog studies that the emesis is mediated in large part, through the central nervous system. The compound is also a gastro-intestinal irritant. Dehydration, most likely a secondary effect of water and electrolyte loss was observed in the high dose group. The treatment with Ringers solution to replenish the electrolyte loss was effective in reversing the dehydration but was not totally corrective. Food consumption was generally comparable between treated and control groups. Body weights for females in the high dose group were generally depressed during the course of the study and were statistically significantly decreased for males from week 19 thru week 26. It would appear that the loss of weight is either secondary to the effects of emesis and/or diarrhea or a direct systemic effect of unknown definition resulting from a decreased food utilization. Whether or not the causative factor is direct or indirect it appears that the following results are inter-related events -decreased body weight, anemia, decreased albumin, decreased calcium, and increased liver weight. Serum calcium is protein bound primarily to albumin, and here the absence of other evidence to the contrary, the decreased calcium level is probably a secondary effect to the decreased serum albumin although other probabilities can not be totally exlcuded. The serum albumin levels may be secondary to the anemia in that the regeneration of hemoglobin protein has priority over that of plasma protein. The decreased body weights and increased liver weight (histological examination of liver tissue showed that the tissue was normal) may also be reflective of protein metabolism irregularities or imbalance to some extent, although fluid loss and imbalance within the organism certainly can not be ignored. The extent to which the skin lesions contributed to the dehydration is not known but was probably minor by comparison to the diarrhea and emesis. It is also pointed out here that no evidence of hemo-concentration was evident thus further substantiating the case for anemia. Neurological findings appeared to be comparable between groups. Any differences which might exist could be attributed to electrolyte imbalance or a generalized depression. The examination of the spleen revealed

no differences from controls at 180 days contrary to what was observed at 90 days.

One female animal in the low dose group (2.0 mg/kg) manifested a skin lesion. It is this reviewer's opinion that this effect at this level is either dose related or compound related, with the effect occurring more often at the higher doses but in a randomly distributed manner. This position is taken in view of the number of lesions observed at the higher levels, the duration of the lesions, the animal behavioral response reported (i.e., observers' comments), location of the lesion and a seeming correlation between open sores of the neck and right hind paw which was observed in some animals.

These skin lesions are either a result of systemic toxicity or the result of direct topical exposure to the test article, leading to itching, scratching and subsequent formation of lesions. Topical exposure may be considered through animal contact of its own eresis and feces which would litter and contaminate the animal itself or the cage floor. Male dogs did not show lesions of the skin at a dose level of 2.0 mg/kg.

Conclusion: The dose of 2.0 mg/kg/day can not be considered, at this time, a definitive NOEL for this study and can not at this time be considered supportive of permanent tolerances, unless some rationale is presented that the skin lesions are the result of a non-systemic effect. If the question of the skin lesions is resolved, a no-observable-effect-level could not be established until the question of the spleen weight at 90 days is resolved.

If the question of the skin and spleen weight is resolved the NOEL for the six-month dog study could be established at 5.0 mg/kg/day.

Lowest Effect Level: 2.0 and 5.0 mg/kg/day based upon skin lesions; decreased spleen weight at 90 days at all dose levels (2.0 mg/kg/day assumed) both sexes.

No-observable-effect-level: less than 2.0 mg/kg/day (see conclusion).

Classification: Core-Guideline.

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Page _____ is not included in this copy.

Pages 130 through 132 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

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Subject: 28 Day Dietary Study - Dairy Cattle: (1) The primary purpose of this study was to determine if lactating dairy cows fed fluvalinate technical for a period of 28 days and followed by a 14 day withdrawal period would leave fluvalinate residues in the milk, liver, kidney, muscle, blood and fat of dairy cattle. (2) This study was also viewed by the sponsor as a subchronic dietary toxicity study as well. This consideration by the sponsor is viewed by this Toxicology Branch reviewer as ancillary to the primary purpose of the study.

Test Compound: ZR-3210 Technical (Racemic Mixture), Mavrik® Technical

Accession No. 070096

Test Facility: Kearley and Young Research
Turlock, California

Study No. Not given

Responsible Professionals:

Edward O. Kearley, D.V.M.
Robert Young Jr., D.V.M.

Testing Period: September 2 - October 13, 1980

Report Submitted to Sponsor: January 27, 1981

Purity of Test Material: Not given

Homogeneity Stability and Concentration Analysis in Feed: Not given, but apparently conducted.

Materials, Methods, Summary, and Conclusions:

Three groups of lactating Holstein cows, ranging from 3-9 years of age, consumed the following doses of fluvalinate for 28 consecutive days, 0.145, 0.58 and 2.32 mg/kg/day. Two additional cows served as untreated controls. These daily dosages were achieved by blending fluvalinate at 40, 160 and 640 ppm into a small quantity of feed which was referred to as the pre-mix. The remainder of the required daily food intake was provided as untreated feed only after the treated feed had been totally ingested. The cows were fed three times per day. The amount of fluvalinate pre-mix fed was adjusted to the cows body weight change on day 21.

During the 28 days of dosing, all cattle were observed for general health, appearance and behavior. Food consumption was recorded daily and body weights weekly. Animals were milked 3 times a day and samples were taken once every 4 days. Milk production was recorded daily.

It was reported that the investigator saw no indication of an adverse effect attributable to fluvalinate ingestion at any dose level based on general observations, increase in weight gain or persistence of milk production.

A 2 week withdrawal period followed the last administration of pre-mix.

Seven cows were slaughtered on day 28, three cows on day 35 and four cows were slaughtered on day 42.

Gross necropsy revealed no pathology attributable to treatment. Four of the fluvalinate treated cows were noted at sacrifice to be pregnant (varying from 98-203 days of gestation) and carried a total of 5 fetuses. All fetuses appeared normal upon examination.

It was therefore concluded that fluvalinate under the conditions of the test appeared to be without adverse effects at levels as high as 640 ppm.

Classification: Supplementary

Note: It is also noted here for possible future reference that urine and feces samples were also taken (see Residue Chemistry Branch review of PP 1G2520/FAP 1H5304).

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